Journal of Chromatography, 643 (1993) 227–238
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2729

Determination of chlorophenoxy and other acidic herbicide residues in ground water by capillary gas chromatography of their alkyl esters formed by rapid derivatization using various chloroformates

S. Butz and H.-J. Stan*

Institute of Food Chemistry, Technical University of Berlin, Gustav-Meyer-Allee 25, W-1000 Berlin 65 (Germany)

ABSTRACT

A simple method for the determination of several chlorophenoxy acid and other acidic herbicides as methyl, ethyl and butyl esters by means of capillary gas chromatography is described. Derivatization with chloroformates (methyl, ethyl or butyl chloroformate) to give the corresponding methyl, ethyl or butyl esters of the pesticides tested can easily be achieved. Fifteen chlorophenoxy acid and seven other acidic herbicides can easily be determined by gas chromatography with electron-capture or mass spectrometric detection at the relevant residue levels of 100 ng/l in water samples. Recoveries were determined with spiked tap and ground water samples at 50, 100 and 200 ng/l using solid-phase extraction with RP-18 material. Nineteen out of the 22 herbicides could be found with a recovery of more than 75% and the remaining three herbicides could be extracted with a recovery of more than 50%. Tentative confirmation can be achieved by simply esterifying two aliquots of the sample extracts with two different chloroformates.

INTRODUCTION

Phenoxy acid herbicides are in widespread use for weed control, which results in their presence as residues in surface and ground waters. Various methods for their determination have been published [1–4] and a comprehensive critical treatment of the methods available up to 1980 was given by Sirous *et al.* [5].

In recent years, solid-phase extraction (SPE) has grown in popularity and gained a reputation as a reliable method for the extraction of pesticide residues from water samples [6,7]. Phenoxy acid herbicides were also found to be extracted with good yields after acidification of the water samples to pH 2. Solid-phase extraction and elution are usually followed either by high-performance liquid chromatography (HPLC) with UV or photoconductivity detection [8] or by gas chromatography (GC) with electron-capture detection (ECD) [6,7,9–11]. Using GC–ECD, derivatization is necessary in order to reach the level of 100 ng/l per substance which has been fixed by the European Community.

The most common derivatives are the methyl esters, using, for example, H_2SO_4–methanol, diazomethane, BF_3–methanol or dimethyl sulphate for methylation [10,11]. Derivatization with pentafluorobenzyl bromide produces derivatives that exhibit very good ECD responses [10–13]. The drawback of this derivatization method, however, is the lack of selectivity of pentafluorobenzyl bromide, leading to the derivatization of a large number of matrix compounds. The results are very complex chromatograms, which make identification and quantification very difficult.

Chloroformates have been known as a possible source of mixed anhydrides since the beginning of this century [14]. In 1990, Husek *et al.* [15] published a rapid derivatization procedure for fatty acids using

* Corresponding author.

0021-9673/93/$06.00

chloroformates as reagents. The method was subsequently extended to the derivatization of hydroxycarboxylic acids [16] and amino acids [17]. These chloroformates seem to be promising for the derivatization of acidic herbicides because of the simplicity of sample preparation. In this paper, a procedure for the derivatization of acidic herbicides using methyl, ethyl and butyl chloroformate to produce methyl, ethyl and butyl esters, respectively, is described.

EXPERIMENTAL

Materials

All pesticide standards were of analytical purity, purchased from Promochem (Wesel, Germany), or of Pestanal quality, from Riedel de Haën (Seelze, Germany). Sample vials, screw-caps and septa were purchased from Zinsser (Frankfurt, Germany) and 200-μl inserts for the sample vials were obtained from CS-Chromatographie Service (Langerwehe, Germany).

Stock solutions of all compounds were prepared in toluene or methanol. Standards and samples were finally dissolved in toluene. All solvents were Pestanal products from Riedel de Haën. Ethyl chloroformate, methyl chloroformate, butyl chloroformate and HCl were purchased from Merck (Darmstadt, Germany). Solid-phase extraction cartridges (6 ml) (polypropylene) and RP-18 material were obtained from Baker (Frankfurt, Germany). Adjustable Transferpettors (1–10 and 10–100 μl) were supplied by Brand (Wertheim, Germany).

The following reaction mixtures were used: (A) acetonitrile–ethanol–water–pyridine (5:2:2:1, v/v); (B) acetonitrile–methanol–water–pyridine (2:2:7:1, v/v); and (C) acetonitrile–butanol–water–pyridine (2:2:6:1, v/v).

Instrumentation

Gas chromatography–mass spectrometry. An HP 5890 gas chromatograph with an HP 5970 mass-selective detector and an HP 59970 MS ChemStation, equipped with an HP 7673 autosampler and a split–splitless injector for capillary columns, was employed.

For GC, a fused-silica capillary column (12 m × 0.20 mm I.D.), coated with SE-54 with a film thickness of 0.32 μm, was used with helium as the carrier gas. The injection port temperature was 210°C and the transfer line temperature 190°C. The column temperature programme was 2.9 min at 90°C, increased at 6°C/min to 240°C and held at 240°C for 20 min. A 1-μl volume of sample was injected by the autosampler using hot splitless injection with the split closed for 1 min.

For MS, the transfer line temperature was 190°C and the ion source temperature 200°C. The scanned mass range was 50–500 u, scan rate 1.22 scans/s and threshold 500. The solvent delay was 10 min. The voltages of the repeller, draw out, ion focus, entrance lens and X-ray and the parameters for the quadrupole mass filter were set according to the values proposed by the programme AUTOTUNE, which automatically optimizes these parameters using perfluorotributylamine (PFTBA) as a calibration standard.

Gas chromatography with electron-capture detection. An HP 5890 gas chromatograph with an electron-capture detector, an HP 7673 autosampler and a split–splitless injector for capillary columns was employed. Nelson analytical Software 2600 was used for data acquisition.

A fused-silica column (25 m × 0.20 mm I.D.), coated with SE-54 material with a film thickness of 0.32 μm, was used with helium as the carrier gas. The injection port temperature was 220°C and the detector temperature 300°C. The column temperature programme was 1 min at 100°C, increased at 30°C/min to 150°C, held for 2 min, then increased at 3°C/min to 205°C and at 10°C/min to 260°C, held at 260°C for 25 min. A 1-μl volume of sample was injected with the autosampler using hot splitless injection with the split closed for 1 min.

Sample preparation

A 1-l water sample was spiked with a mixture of pesticides to achieve a concentration of 100 ng/l per substance. The internal standard, 2,4-dichlorobenzoic acid, was added at the same concentration level. The sample was then acidified to pH 1.5 with HCl. Each SPE cartridge was filled with 1 g of RP-18 adsorbent. Conditioning was performed successively with 5 ml of acetone, 5 ml of methanol, 10 ml of distilled, deionized water and finally 5 ml of water acidified to pH 1.5. The solvents were drawn through the cartridges by means of a gentle vacuum and the cartridge was not permitted to run dry after

addition of the acidified water. The water sample spiked with the herbicides was then percolated through the cartridge at a flow-rate of *ca.* 8 ml/min. After drying the cartridge for 2–3 h under a gentle stream of nitrogen, the herbicides were eluted with 2 ml of methanol. The eluate was dried under a gentle stream of nitrogen.

Hydrolysis of acidic esters

As the pesticides diclofop, fenoxaprop and quizalofop are not available as free acids, they were prepared by alkaline hydrolysis. The ester was dissolved in a solution of ethanol (10 ml) and aqueous potassium hydroxide (40%, 30 ml) and the mixture was heated under reflux for 5 h. After cooling, the pH was adjusted to 2 with hydrochloric acid. The pesticide was then extracted with three portions of ethyl acetate (40 ml) in a separating funnel. The combined extracts were dried over anhydrous sodium sulphate and decanted into a 250-ml round-bottomed flask. The solvent was removed under vacuum with a rotary evaporator and the residue treated as described below.

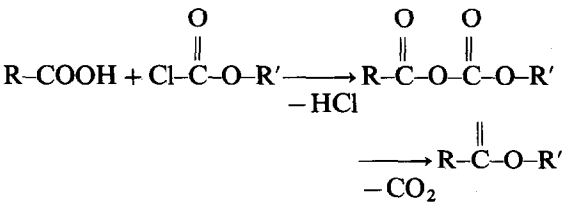
Derivatization

The dry sample extract was placed in a reaction tube and dissolved in 100 μl of reaction mixture A followed by 7 μl of ethyl chloroformate to obtain the corresponding ethyl esters. To obtain the methyl esters, the dry sample eluate was dissolved in 100 μl of reaction mixture B followed by 7 μl of methyl chloroformate. To obtain the butyl esters, the dry sample eluate was dissolved in 100 μl of reaction mixture C followed by 7 μl of butyl chloroformate. The reaction tube was shaken gently for about 5 s, preferably against a pad, to initiate the gas evolution that can usually be observed. This is caused by decomposition of the reagent to alcohol and carbon dioxide. The derivatives were dried with a gentle stream of nitrogen and finally dissolved in 100 μl of toluene, from which an aliquot of 1 μl was injected into the GC–ECD or GC–MS system.

RESULTS AND DISCUSSION

The known simplicity of derivatizing fatty acids, hydroxy acids and amino acids in the field of clinical chemistry, as demonstrated by the work of Husek and co-workers [15–17], encouraged us to investigate whether chloroformates would also be suitable for the esterification of the carboxyl function of chlorophenoxy acid herbicides and similar acidic herbicides. Our attention was attracted by the possibility of producing parallel series of alkyl esters with the same sample extract. This would enable the analyst to make a first check for the presence of one of these pesticides by comparing the parallel chromatograms with the corresponding retention time tables. A second obvious advantage of parallel derivatization is that if the usually employed methyl ester of one of these pesticides is overlapped by a matrix compound it is not likely that the same situation would occur with its ethyl or butyl ester. The pesticides under investigation are shown in Fig. 1.

The reaction scheme is described by the following equation:



$R' = CH_3$, CH_2CH_3, $(CH_2)_3CH_3$

To find the optimum conditions for the derivatization procedures, pyridine, triethylamine and sodium carbonate were tested as catalysts. Only with pyridine was an instantaneous evolution of carbon dioxide gas observed and a single product (methyl, ethyl or butyl ester) was found to be formed. These findings confirm the observations of Husek *et al.* [15], who studied extensively the reaction conditions to form methyl, ethyl and 2-chloroethyl esters with fatty acids. Using triethylamine or sodium carbonate, the equivalent ester was also formed but together with some by-products. The compositions of reaction mixtures A, B and C had also to be varied in order to find optimum derivatization conditions.

This detailed investigation resulted in the procedure described above. The surprising finding was that with a constant alcohol-to-pyridine ratio of 2:1 the optimum proportions of acetonitrile and water varied with the different chloroformates as reported. Our optimum for the formation of methyl esters did not exactly confirm the reaction procedure proposed

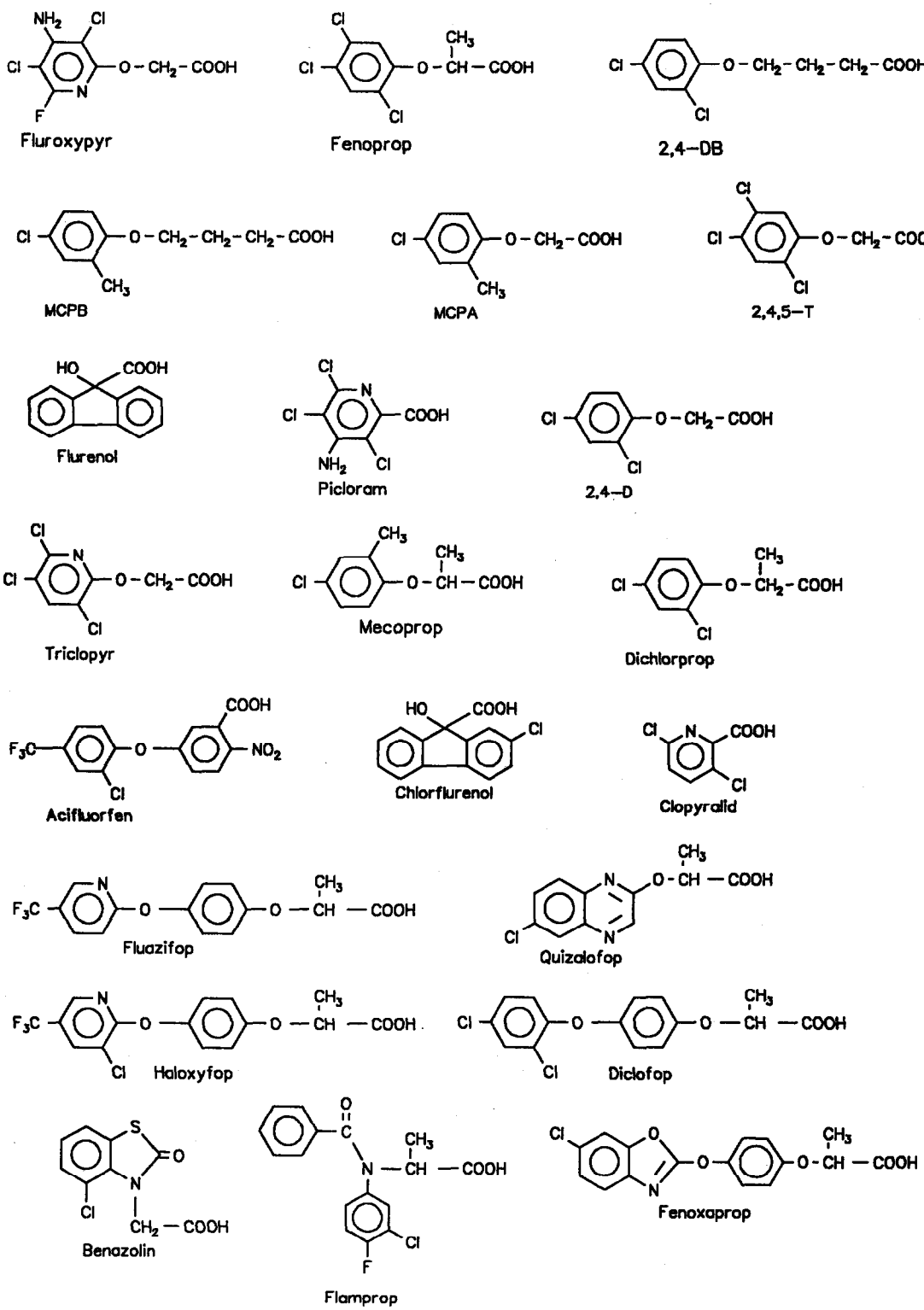


Fig. 1. Structures of pesticides investigated.

by Husek *et al.* [15]. It must be noted, however, that our acids are of a different nature to the aliphatic fatty acids that they investigated. All the pesticides shown in Fig. 1 were derivatized to obtain methyl, ethyl and butyl esters. With all the pesticides the esters were produced in good yields.

Chromatograms of a mixture of pesticides obtained with the GC–ECD system are shown in Fig. 2. All the pesticides of the mixture can be readily recognized. Each group of esters exhibits the same sequence pattern. Although the sequence of the alkyl esters is the same with all alkyl moieties, different response ratios were observed. This is particular evident with the later eluting peaks 9–13. The variation of response factors depends on the structures of the individual pesticides (Fig. 1), but an influence by the alkyl ester group should not be expected. The varying peak sizes rather reflect the reproducibility of the derivatization of the compounds at the 100-ng level. With methyl esters, the first-eluting peak, the internal standard 2,4-dichlorobenzoic acid, is always found to be very small, indicating losses due to volatility. This is to be expected, because 2,4-dichlorobenzoic acid is only used to check the extraction and the derivatization procedure and is not used for any final quantification purposes.

It should be noted that the group of active substances contains herbicides with hydroxyl groups adjacent to the carboxyl group. These two particular herbicides were also found to be completely esterified. Under the conditions described under Experimental, the carboxyl group was found to form the corresponding alkyl ester as for all other acids. The hydroxyl group, however, was esterified to form the alkyl carboxy ester, as indicated by the mass spectral investigation.

Fig. 3 shows the GC–MS traces for flurenol methyl, ethyl and butyl ester. The reactions resulted in two peaks with all three esters. The mass spectra for peaks A and B in Fig. 3 indicate which products were formed during esterification. Peak A represents the esterified carboxylic function, which leads to a molecular ion at m/z 240 for the flurenol methyl ester, at m/z 254 for the ethyl ester and at m/z 282 for the butyl ester. Peak B in Fig. 3 represents flurenol esters in which the hydroxyl function is also esterified. The increase in the molecular ions at m/z 298 for the methyl, m/z 326 for the ethyl and m/z 382 for the butyl ester clearly indicates the formation of the corresponding alkyl carboxy esters. All the spectra show the molecular ion well displayed and the interpretation of the fragmentation pattern is convincing.

To demonstrate the applicability of the method in pesticide residue analysis, tap and ground water samples were spiked with acidic herbicides at a level of 100 ng/l and analysed as described. Screening analyses were carried out by capillary GC–ECD. The chromatograms of the extract of such a spiked ground water sample are shown in Fig. 4. All the pesticides, including the internal standard 2,4-dichlorobenzoic acid, can be identified.

In Table I, the retention times and recoveries of the three alkyl esters of all the pesticides tested are given. These analyses were carried out with the GC systems permanently used in our laboratory for pesticide multi-residue determinations in water and food samples with aldrin as internal standard. The carrier gas flow-rate in the GC–ECD system is always adjusted to give aldrin at 25.00 min. Therefore, the retention times reported in Table I fit in this overall frame. The retention times of all 22 pesticides were found to be very stable for the methyl, ethyl and butyl esters, matching our experience with most of the pesticides determined with the multi-residue method.

The recoveries were better than 75% for most of the pesticides investigated. However, that of clopyralid acid was only 51%, MCPA 63% and picloram 69%, which we do not consider to be satisfactory. Therefore, the recovery study was repeated with increased amounts of 1.5 and 2.0 g of adsorbent, but no better recoveries were obtained. This is the reason for recommending 1 g of RP-18 adsorbent for SPE. The relative standard deviation, however, was found to be acceptable for all pesticides at the low residue levels in ground water samples. It should be emphasized that all 22 herbicides could be easily determined with the method described at the low residue concentration levels.

The retention time differences of *ca.* 1.5 min observed between the methyl and ethyl esters, *ca.* 4.5 min between the methyl and butyl esters and *ca.* 3 min between the ethyl and butyl esters can be used to confirm the presence of a supposed pesticide. The sample extract is simply split and the aliquots derivatized to produce two or even all three of the alkyl esters.

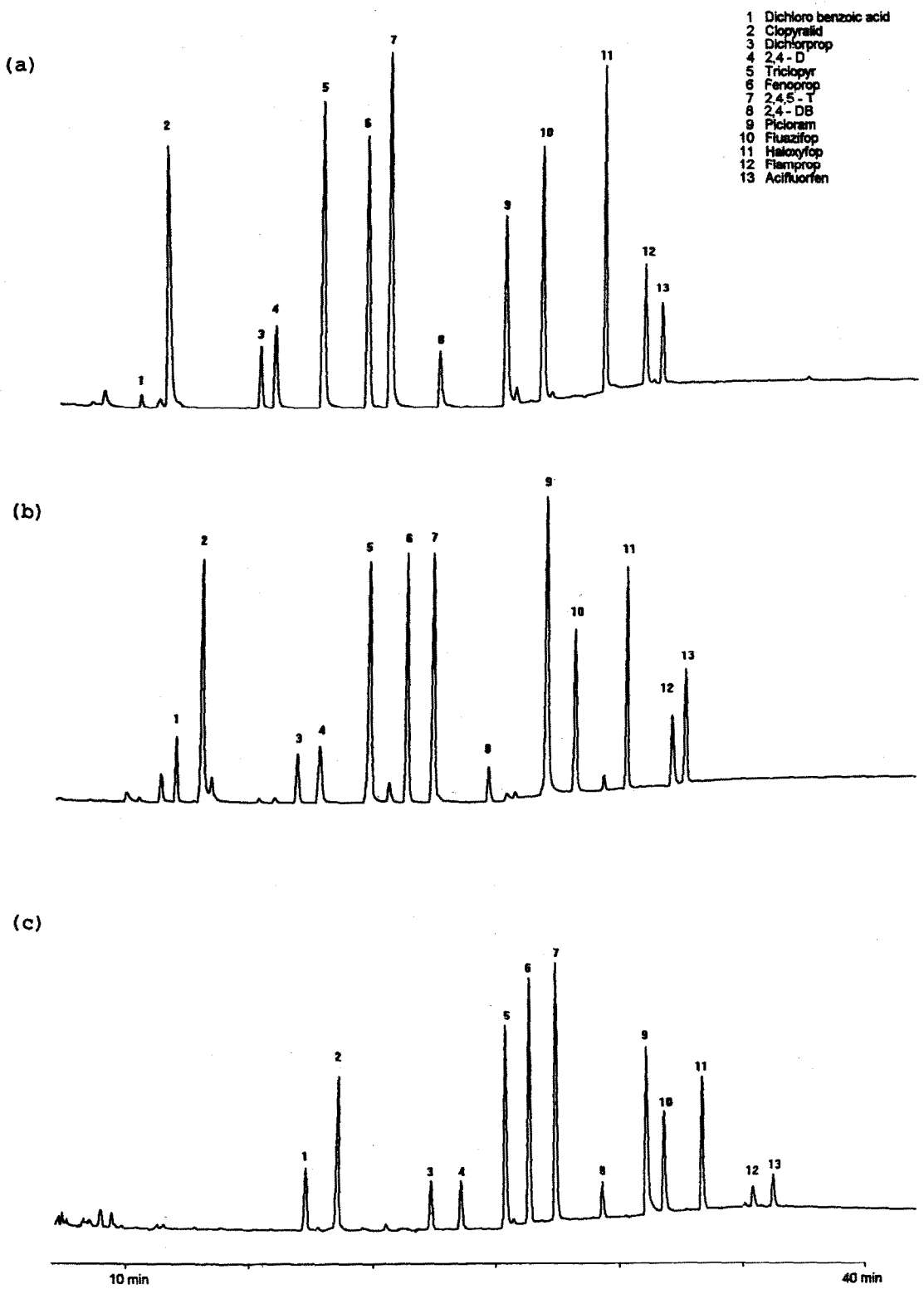


Fig. 2. Gas chromatograms of a standard mixture of thirteen acidic herbicides after derivatization to the (a) methyl, (b) ethyl and (c) butyl esters. Amount injected into the GC–ECD system = 1 ng. Peaks: 1 = dichlorobenzoic acid; 2 = clopyralid; 3 = dichlorprop; 4 = 2,4-D; 5 = triclopyr; 6 = fenoprop; 7 = 2,4,5-T; 8 = 2,4-DB; 9 = picloram; 10 = fluazifop; 11 = haloxyfop; 12 = flamprop; 13 = acifluorfen.

To demonstrate the practical use of this method, ground water samples were spiked each time at 100 ng/l with only one pesticide unknown to the analyst. An example chromatogram of a methylated extract is shown in Fig. 5a and that of the corresponding ethylated extract in Fig. 5b. According to the retention time table, the marked peak at 17.13 min was found to be triclopyr methyl ester. Ethylation of the second half of the sample extract showed that the peak of interest was shifted to the expected retention time of 19.02 min. The pesticide in the sample was identified as triclopyr, which could easily be confirmed by GC–MS. Fig. 5a and b indicate that most of the matrix compounds seen in

(a) Flurenol after methylation

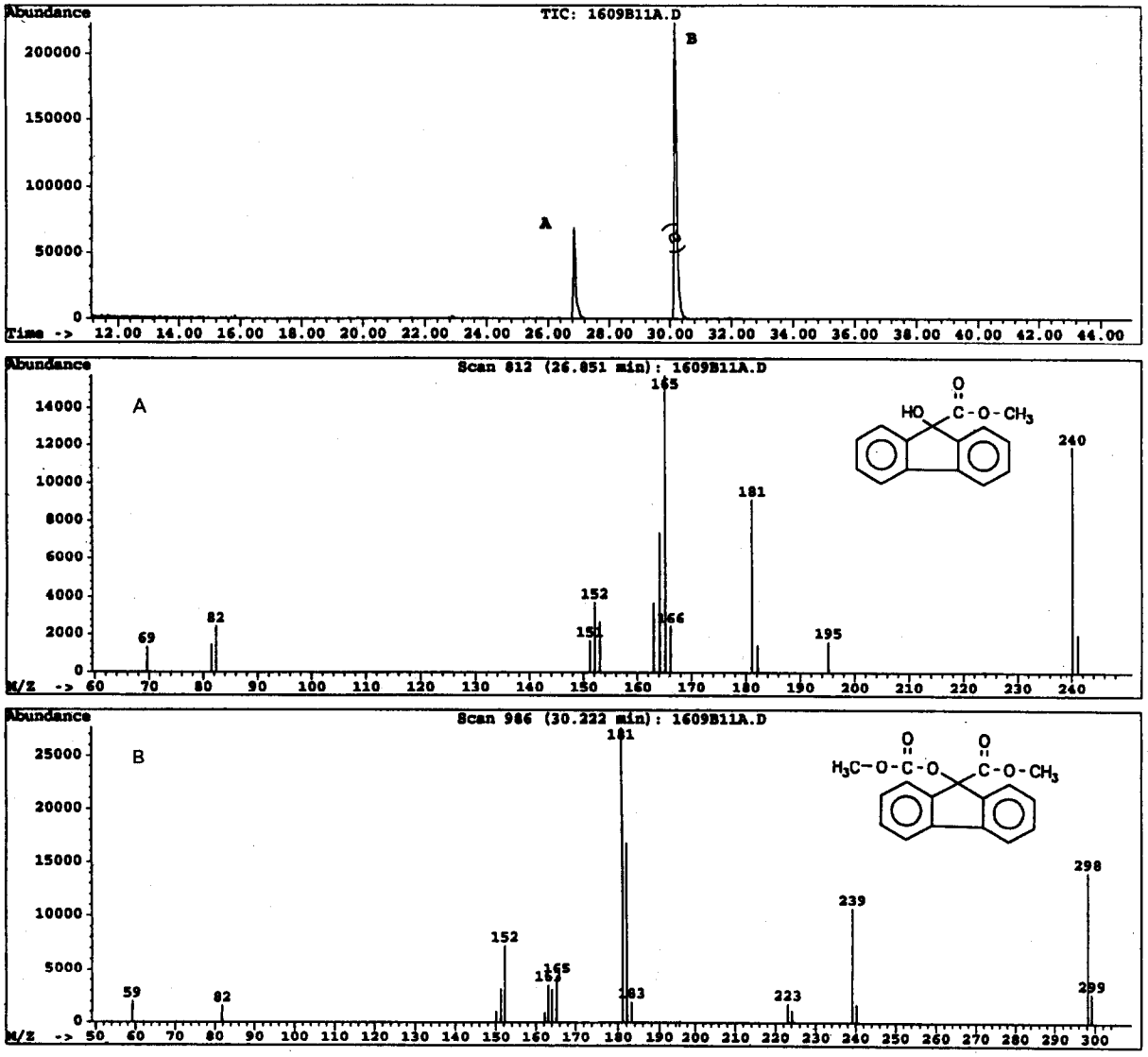


Fig. 3.

(Continued on p. 234)

the chromatograms obtained with ECD were not shifted when two different esterifications were applied, an indication that those peaks do not represent esters formed during derivatization. This is the reason why, in the samples treated as described, the acidic herbicides could always be easily seen.

To summarize, it can be stated that the determination of trace levels of 22 acidic herbicides can be achieved using these simple derivatization procedures. The obvious advantage of the use of chloroformates is the ease wiht which a tentative confirmation can be carried out by simply esterifying two aliquots of the extract with two different chloroformates. The described confirmation, however,

(b) Flurenol after ethylation

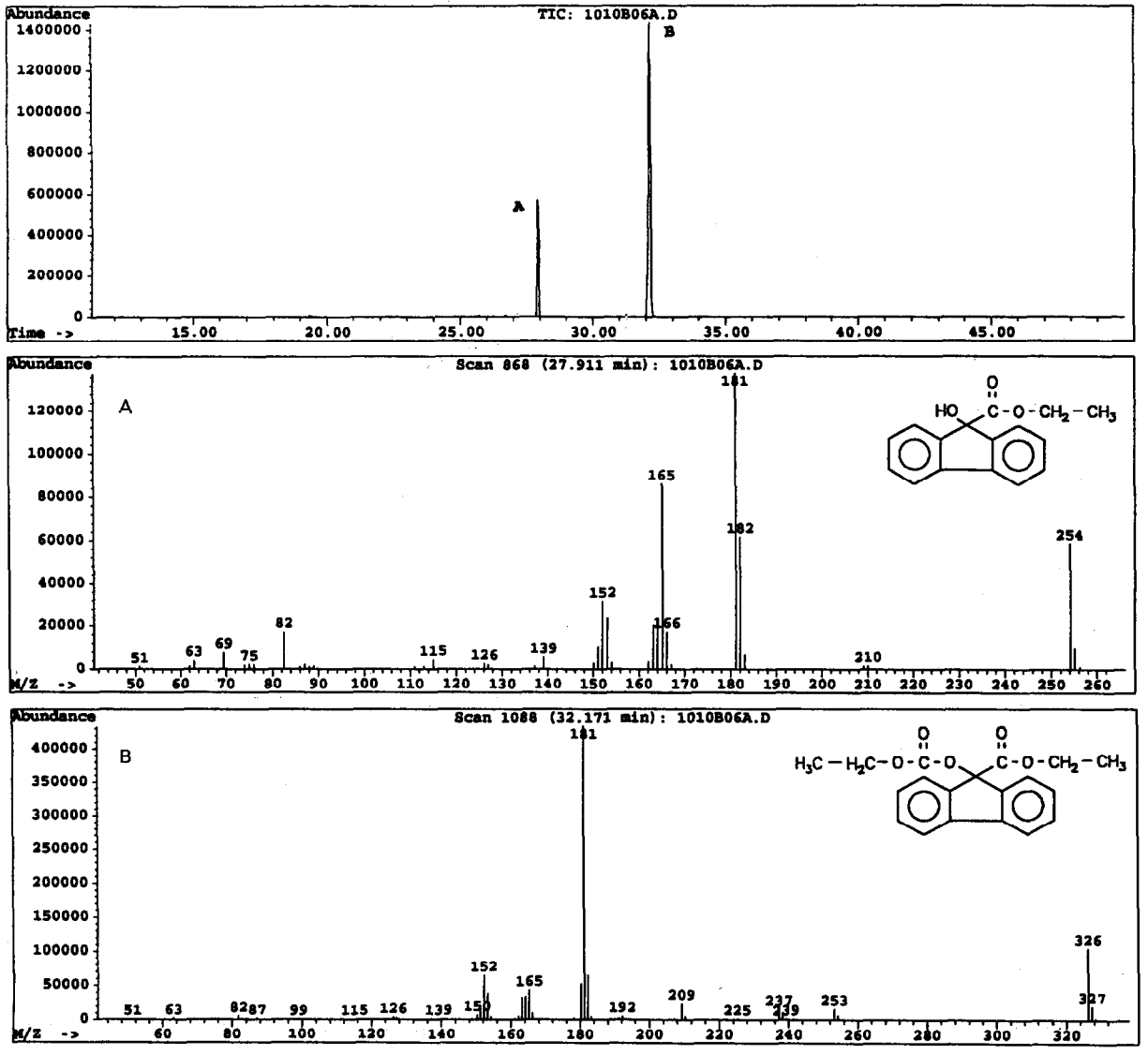


Fig. 3.

proves only that a peak shifting in the way described is produced by a substance that can be derivatized with chloroformates, in the first aliquot to the methylated and in the second to the ethylated or butylated derivative, for example. An unequivocal confirmation, however, needs the use of GC–MS.

CONCLUSIONS

With the simple derivatization procedure described, 22 acidic herbicides can be converted into the corresponding methyl, ethyl or butyl esters, which are then amenable to capillary GC. The

(c) Flurenol after butylation

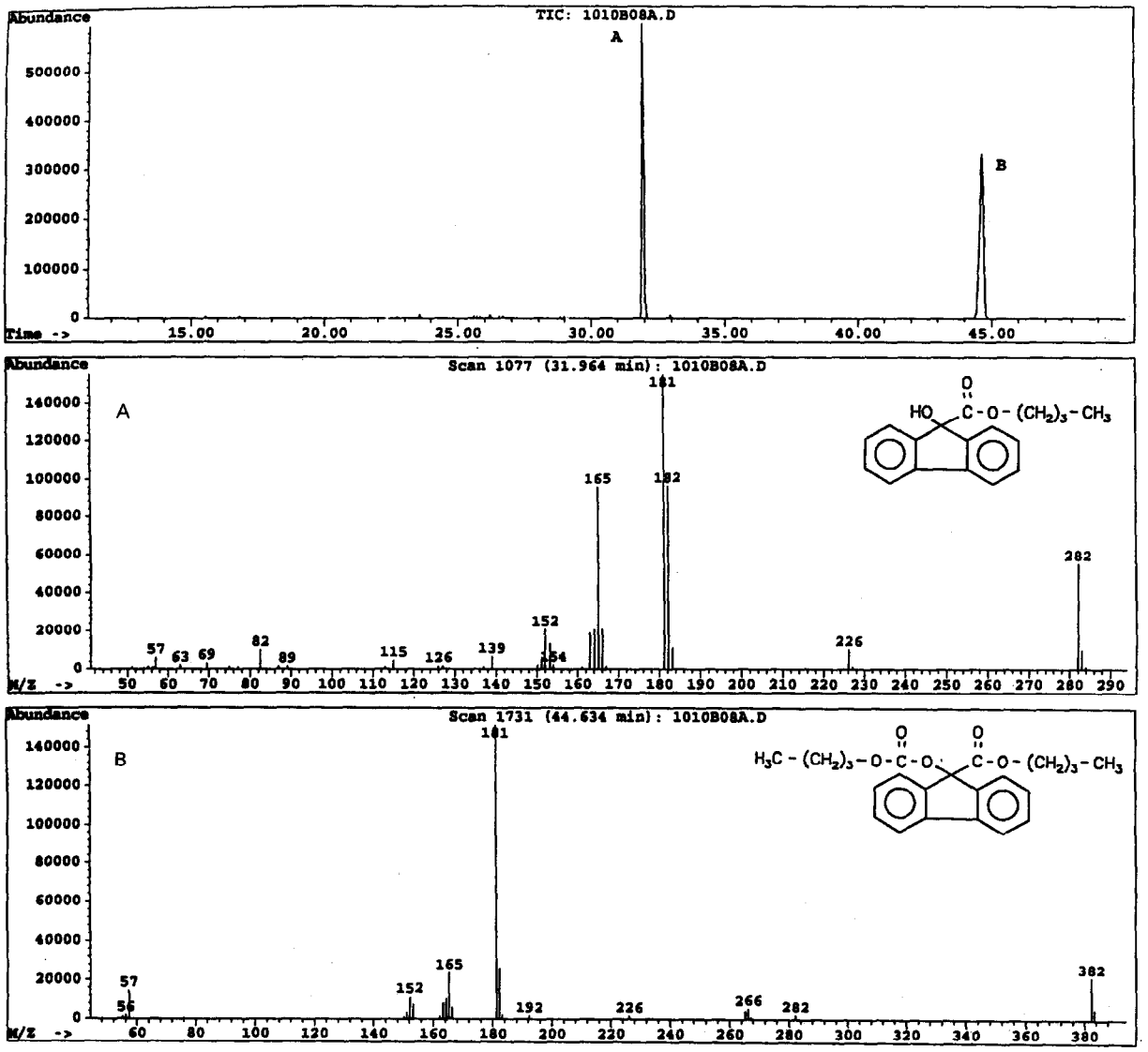


Fig. 3. TIC and mass spectra of flurenol after (a) methylation, (b) ethylation and (c) butylation.

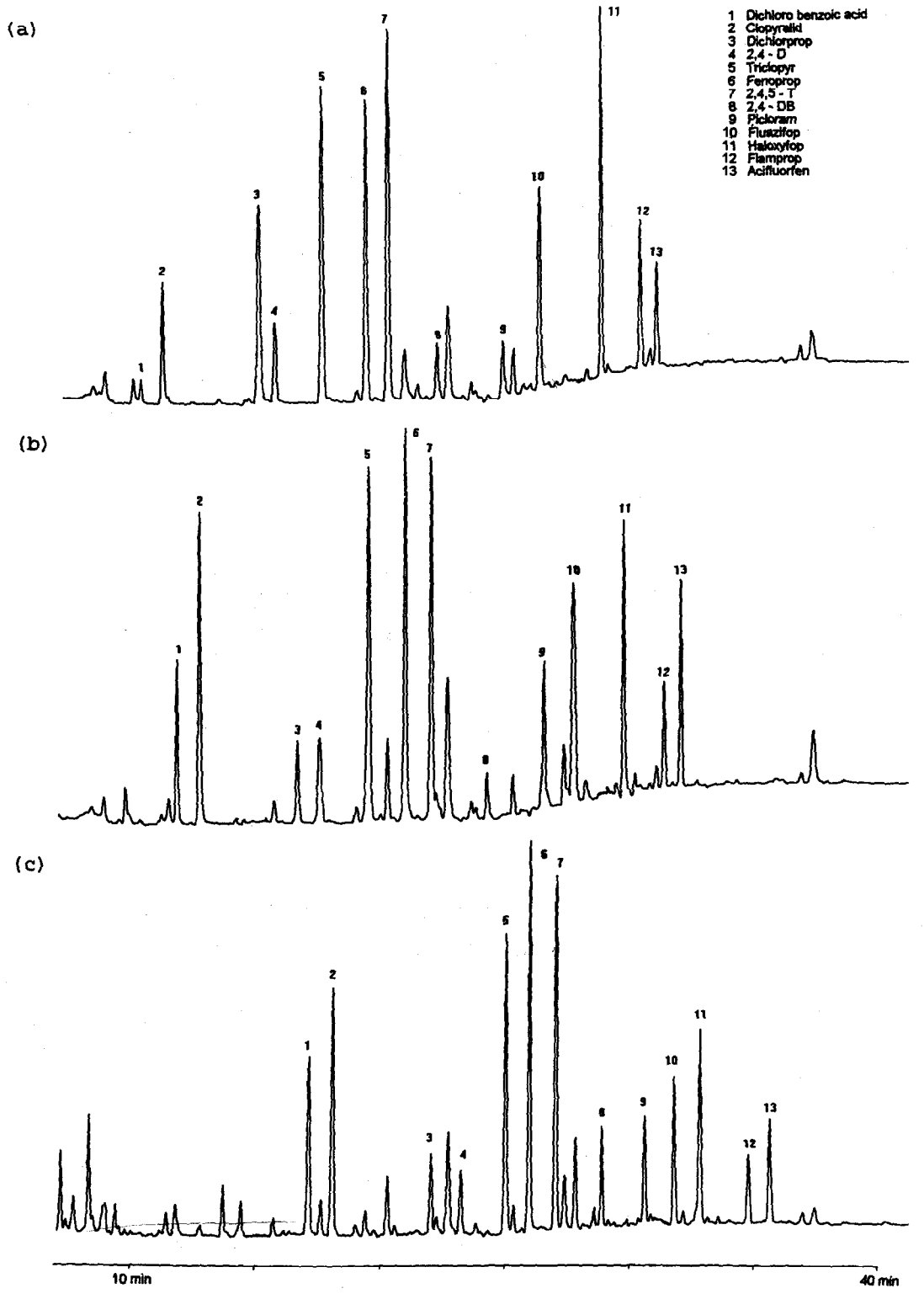


Fig. 4. Gas chromatogram of a ground water sample spiked with 100 ng/l of a mixture of thirteen acidic herbicides derivatized to their (a) methyl, (b) ethyl and (c) butyl esters. Detection: ECD. Peaks as in Fig. 2.

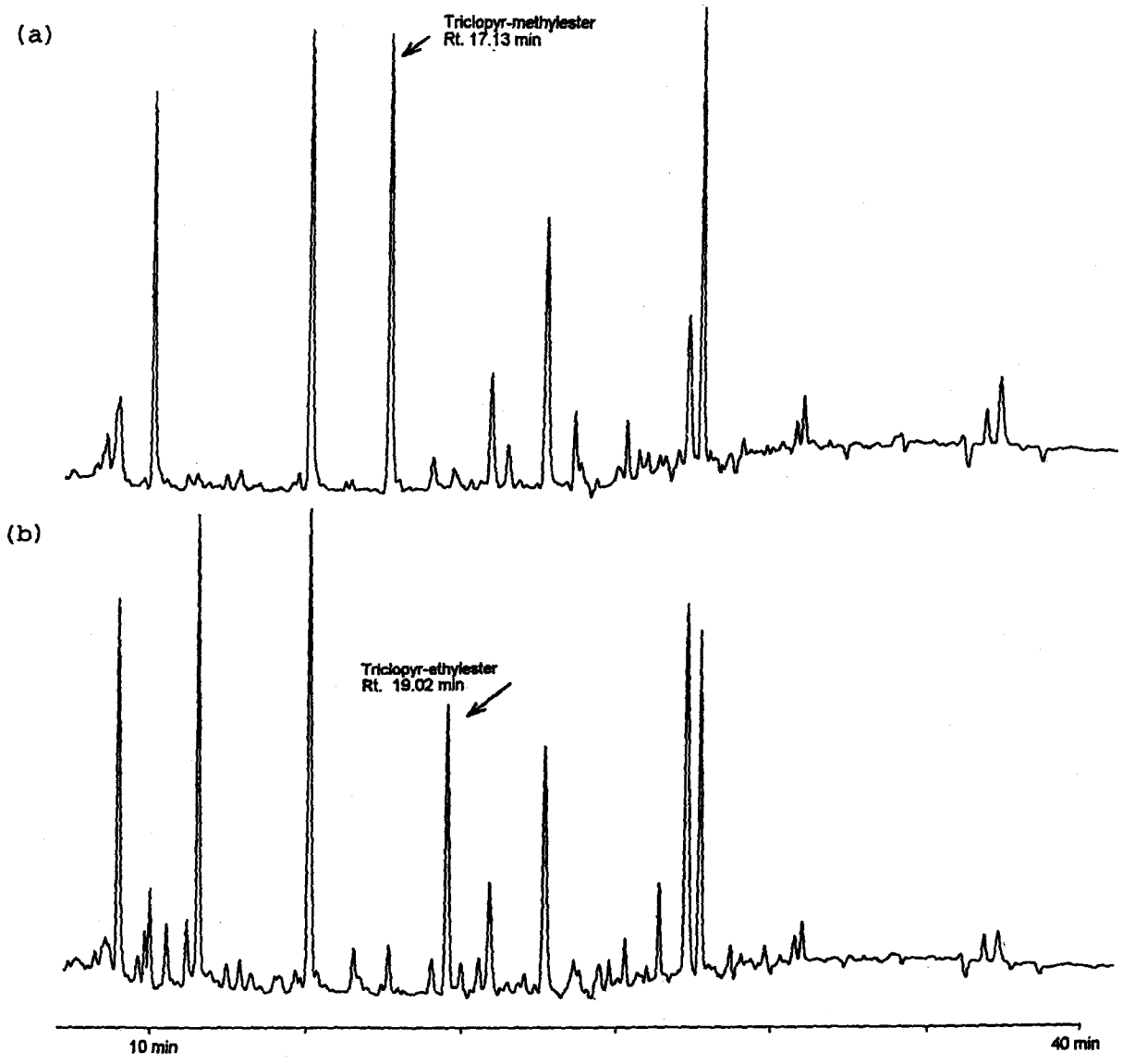


Fig. 5. Gas chromatogram of a ground water sample after derivatization to the (a) methyl and (b) ethyl esters. Detection: ECD.

derivatives are highly specific for their parent compounds. The preparation of a set of ten samples for GC takes about 10 min and requires only small amounts of inexpensive reagents. Almost without additional laboratory work a second set of derivatives can be prepared, allowing a tentative confirmation of suspected pesticide residues. Therefore, we consider that this derivatization method is unrivalled in ease, speed and flexibility for the preparation of alkyl esters.

TABLE I

RETENTION TIMES AND RECOVERIES OF THE ALKYL ESTERS OF 22 ACIDIC HERBICIDES

GC–ECD, 50 m × 0.20 mm I.D. SE-54 column, conditions as described under Experimental. All retention times were measured with the GC system adjusted to give aldrin with a retention time of 25.00 min (see text).

Pesticide	Retention time (min)			Recovery (%)	R.S.D. (%) ($n = 10$)
	Methyl ester	Ethyl ester	Butyl ester		
2,4-Dichlorobenzoic acid (ISTD)	10.15	11.25	16.67	80	4.5
Clopyralid acid	10.98	12.13	17.58	51	6.3
Mecoprop	12.93	14.08	18.50	82	5.9
MCPA	13.35	14.75	19.27	63	7.5
Dichlorprop	14.75	15.85	20.35	98	5.1
2,4-D	15.33	16.73	21.40	83	6.8
Triclopyr	17.13	19.02	22.55	93	7.2
Fenoprop	18.85	19.98	24.28	98	3.9
MCPB	19.53	20.03	23.98	82	5.6
2,4,5-T	19.72	21.15	25.18	88	6.2
Fluroxypyr	21.55	23.18	27.35	77	8.1
2,4-DB	21.63	23.45	27.42	87	6.4
Picloram acid	24.20	25.93	27.98	69	7.1
Benazolin	25.56	26.80	30.35	82	5.7
Fluazifop	25.62	26.92	29.68	83	6.1
Haloxyfop	27.98	28.88	31.82	79	5.4
Flurenol	28.33	29.21	33.30	82	6.3
Chlorflurenol	28.43	29.35	33.39	80	7.5
Flamprop	29.53	30.52	33.73	91	6.5
Acifluorfen	30.53	31.45	34.55	78	5.9
Diclofop	32.77	33.80	36.90	81	6.3
Fenoxaprop	37.53	38.68	41.76	78	5.9
Quizalofop	43.53	44.63	47.68	89	6.1

REFERENCES

1 W. P. Cochrane, *J. Chromatogr. Sci.*, 13 (1975) 246.
2 W. P. Cochrane, *J. Chromatogr. Sci.*, 17 (1979) 124.
3 M. J. Bertrand, A. W. Ahmed, B. Sarrasin and V. N. Mallet, *Anal. Chem.*, 59 (1987) 1302.
4 A. W. Ahmed, V. N. Mallet and M. J. Bertrand, *J. Assoc. Off. Anal. Chem.*, 72 (1989) 365.
5 G. J. Sirous, A. S. Y. Chau and A. E. Smith, in A. S. Y. Chau and B. K. Afghan (Editors), *Analysis of Pesticides in Water*, Vol. 2, CRC Press, Boca Raton, FL, 1982, p. 155.
6 S. H. Hoke, E. E. Brueggemann, L. J. Baxter and T. Trybus, *J. Chromatogr.*, 357 (1986) 429.
7 A. Di Corcia, M. Marchetti and R. Samperi, *Anal. Chem.*, 61 (1989) 1363.
8 W. Schüssler, *Chromatographia*, 29 (1990) 24.
9 C. J. Miles and M. J. Zhou, *J. Agric. Food Chem.*, 38 (1990) 986.
10 W. Weber, in G. Milde and P. Friesel (Editors), *Grundwasserbeeinflussung durch Pflanzenschutzmittel (Schr.-Reihe Verein WaBoLu 68)*, G. Fischer Verlag, Stuttgart, 1987, p. 109.
11 C. Schlett, *Z. Wasser Abwasser Forsch.*, 23 (1990) 32.
12 W. Gilsbach and H.-P. Thier, *Z. Lebensm.-Unters.-Forsch.*, 175 (1982) 327.
13 J. O. DeBeer, C. H. Van Peteghem and A. M. Heyndrickx, *J. Assoc. Off. Anal. Chem.*, 61 (1978) 1140.
14 M. Makita, S. Yamamoto and S. Kiyama, *J. Chromatogr.*, 237 (1982) 279.
15 P. Husek, J. A. Rijks, P. A. Leclercq and C. A. Cramers, *J. High Resolut. Chromatogr.*, 13 (1990) 633.
16 P. Husek, *J. Chromatogr.*, 547 (1991) 307.
17 P. Husek, *J. Chromatogr.*, 552 (1991) 289.