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# Determination of phenoxy ester herbicides by gas and high-performance liquid chromatography

C. SÁNCHEZ-BRUNETE, S. PÉREZ and J. L. TADEO*
*Departamento de Protección Vegetal, CIT–INIA, Aptdo. 8111, 28080 Madrid (Spain)*

## ABSTRACT

The determination of phenoxy ester herbicides was carried out by high-performance liquid (HPLC) and gas chromatography (GC) and their identification by GC–MS spectrometry. Standards of herbicide esters were obtained from the appropriate acid and a suitable alcohol, mainly 2-butoxyethyl, isooctyl or isobutyl alcohol, using acetyl chloride as a catalyst. The mixture was heated at 100°C for 1 h, after cooling 2 ml of acetate buffer (pH 4.6) were added and then the mixture was diluted to volume with methanol. Good ester conversion (>95%) was achieved. The phenoxy esters were analysed by reversed-phase HPLC on a Spherisorb ODS-2 (5-μm) column with acetonitrile–water (85:15) as mobile phase at a flow-rate of 1 ml/min and UV detection at 280 nm. GC was performed on a BP-5 capillary column with helium as carrier gas (10 ml/min) and flame ionization detection. Esters were identified by GC–ion trap detection on a BP-1 capillary column. Several mixtures of phenoxy ester herbicides in formulations were analysed using the proposed methods and good agreement between the HPLC and GC results was obtained.

## INTRODUCTION

Phenoxy acids are an important group of selective herbicides usually formulated in the form of salt or alkyl esters. Analysis of phenoxy ester formulations is based on hydrolysis to the corresponding acid, which is determined by high-performance liquid chromatography (HPLC) [1,2] or gas chromatography (GC) after methylation [3]. Therefore, the acid equivalent and not the ester concentration is determined.

Esterification of phenoxy acids has been reviewed by Cochrane [4] and several workers have carried out derivatization with diazomethane [5], boron trifluoride–methanol [5,6] or fuming sulphuric acid–ethanol [7]. Noble [8] proposed the use of acetyl chloride and the appropriate alcohol to obtain standards of the different phenoxy esters used in commercial formulations. These standards can be used to determine phenoxy esters in pesticide formulations by direct GC analysis.

The aim of this work was to study the determination of phenoxy ester herbicides in formulations by HPLC and GC and their identification by GC–ion trap detection (ITD). The results are compared with those obtained by HPLC determination of the corresponding acids, based on the AOAC method [1].

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## EXPERIMENTAL

### *Instrumentation*

The liquid chromatograph was a Beckman Model 421A equipped with a 20-$\mu$l loop injector, a Model 160A fixed-wavelength UV detector, a Spectra-Physics SP4290 integrator and a Spherisorb ODS-2 ($C_{18}$) column (250 × 4.6 mm I.D.).

A Perkin-Elmer Model 8500 gas chromatograph with a flame ionization detector, an ion trap detector (Finnigan) and a split/splitless injector was employed.

### *HPLC*

*Phenoxy esters.* Acetonitrile–water (85:15) was used as the eluent at a flow-rate of 1 ml/min. The detection wavelength was fixed at 280 nm. The herbicide concentration was calculated by comparing the peak areas obtained for samples with those obtained for standards.

*Phenoxy acids.* Acetonitrile–0.2% acetic acid (40:60) was used at a flow-rate of 1.5 ml/min. The detection wavelength was fixed at 280 nm. The equivalent concentration of esters was calculated by comparing the peak areas obtained for hydrolysed samples with those obtained for the acid standards and then corrected by multiplying by the ratio $MW_{ester}/MW_{acid}$.

### *GC*

A BP-5 fused-silica column (12 m × 0.53 mm I.D.) with a film thickness of 1 $\mu$m was used with helium as the carrier gas at a flow-rate of 10 ml/min and flame ionization detection. The temperature programme was 180°C, held for 5 min, increased at 25°C/min to 250°C, held for 10 min. A 1-$\mu$l volume of sample was injected and the herbicide concentration was calculated by comparing the peak areas obtained for samples with those obtained for the ester standards.

Identification was carried out by GC–ITD with a BP-1 capillary column (12 m × 0.22 mm I.D.) and helium as carrier gas at a flow-rate of 10 ml/min. The temperature programme was 85°C, held for 5 min, increased 20°C/min to 250°C, held for 5 min. A 2-$\mu$l volume was injected with the split closed for 1 min.

### *Mass spectrometric acquisition parameters*

The following conditions were used: transfer line temperature, 250°C; mass range, 40–350 dalton; scan rate, 0.5 s per scan, 2-$\mu$scans; r.f. voltage, 1.1 MHz and 0–7.5 kV; automatic gain control from 78 $\mu$s to 25 ms; solvent delay, 3 min.

### *Materials*

Phenoxy acids were obtained as test substances from several manufacturers: 2,4-D [(2,4-dichlorophenoxy)acetic acid] from Condor (Middlesex, U.K.); 2,4-DP [(2,4-dichlorophenoxy)propionic acid] from BASF (Ludwigshafen, Germany); MCPA [(4-chloro-2-methylphenoxy)acetic acid] and MCPP [(4-chloro-2-methylphenoxy)propionic acid] from Azko (Rotterdam, The Netherlands).

### *Preparation of standard solutions*

*Esters.* A mixture (1:5) of acetyl chloride and a suitable alcohol was prepared, previously cooling the alcohol in an ice-bath. A 2-ml volume of the mixture was added

to 50 mg of the phenoxy acid and the resulting mixture was then heated at 100°C in a sand-bath for 1 h. After cooling to room temperature, 2 ml of acetate buffer (pH 4.6) were added and the solution was transferred to a 100-ml volumetric flask with methanol.

*Acids.* A 50-mg amount of the phenoxy acid was dissolved in 100 ml of methanol.

*Ester hydrolysis.* A 100-mg amount of the corresponding phenoxy ester, or the equivalent amount of the formulated herbicide, was dissolved in 50 ml of methanol and 20 ml of 5 *M* potassium hydroxide solution. After reaction for 1 h, the mixture was acidified to pH 6 with 10 *M* acetic acid (*ca.* 0.8 ml) and diluted to the final volume (100 ml) with methanol.

## RESULTS AND DISCUSSION

The different esters of the phenoxy acids were obtained by the procedure described above. The ester conversion was >95% in all instances, which is in agreement with the results obtained by Noble [8].

The determination of phenoxy esters was accomplished by HPLC and GC under the conditions given above and their retention times are shown in Table I. Direct GC analysis of phenoxy esters in formulations has been carried out previously by other workers [8–10]. However, the determination of these compounds by HPLC has usually been done after hydrolysis to the acids, using different alkyl-silica columns and buffered mobile phases [1,2,11,12]. In this work, an alternative HPLC method for the determination of these herbicides as esters was developed. Although UV detection at 220 nm showed a sensitivity about five times higher, the response at 280 nm produced a cleaner baseline and was selected for the determination. Under these conditions, the detection limit was about 20 ng for each phenoxy ester. These compounds were also determined by HPLC after hydrolysis to the free acids (Table II). The sensitivity obtained with this procedure, which is based on the AOAC method [1], was about 50% lower than that achieved with the proposed HPLC method for the esters. Some representatives chromatograms are shown in Fig. 1.

Individual phenoxy esters have been identified previously by capillary GC on the basis of their retention times [8]. In this work, the identification of the phenoxy esters

TABLE I

RETENTION TIMES OF PHENOXY ESTERS DETERMINED BY HPLC AND GC

HPLC: column, Spherisorb ODS-2 ($C_{18}$) (250 × 4.6 mm I.D.); mobile phase, acetonitrile–water (85:15); flow-rate, 1 ml/min. GC: column, BP-5 (12 m × 0.53 mm I.D.); carrier gas, helium at a flow-rate of 10 ml/min.

| Ester | Retention time (min) | |
|---|---|---|
| | HPLC | GC |
| 2,4-D 2-butoxyethyl | 5.12 | 7.37 |
| 2,4-D isobutyl | 5.15 | 3.75 |
| 2,4-DP 2-butoxyethyl | 6.28 | 7.08 |
| MCPA 2-butoxyethyl | 5.26 | 6.89 |
| MCPP 2-ethylhexyl | 14.10 | 6.73 |

TABLE II

RETENTION TIMES OF PHENOXY ACIDS DETERMINED BY HPLC

Column, Spherisorb ODS-2 ($C_{18}$) (250 × 4.6 mm I.D.); mobile phase, acetonitrile–0.2% acetic acid (40:60); flow-rate, 1.5 ml/min.

| Acid | Retention time (min) |
|---|---|
| 2,4-D | 7.50 |
| 2,4-DP | 11.27 |
| MCPA | 7.73 |
| MCPP | 12.00 |

was carried out by GC–ITD. The mass spectra of these compounds show a molecular ion with high relative abundance in all instances [13,14], which for MCPA and MCPP esters represents the base peak of the spectrum. For the 2-butoxyethyl esters a remarkable peak is observed at $m/z$ M − 73 caused by the loss of the $-O(CH_2)_3CH_3$ fragment. The spectra of 2,4-D esters show the base peak at $m/z$ 57 ($C_4H_9^+$) or $m/z$ 41 ($C_3H_5^+$), which correspond to the alcohol portion of the ester; these ions are also present in the spectra of MCPA and MCPP esters. All the phenoxy esters (RCOOR') show the $R^+$ ion. Fig. 2 shows the mass spectra of some 2,4-D esters.

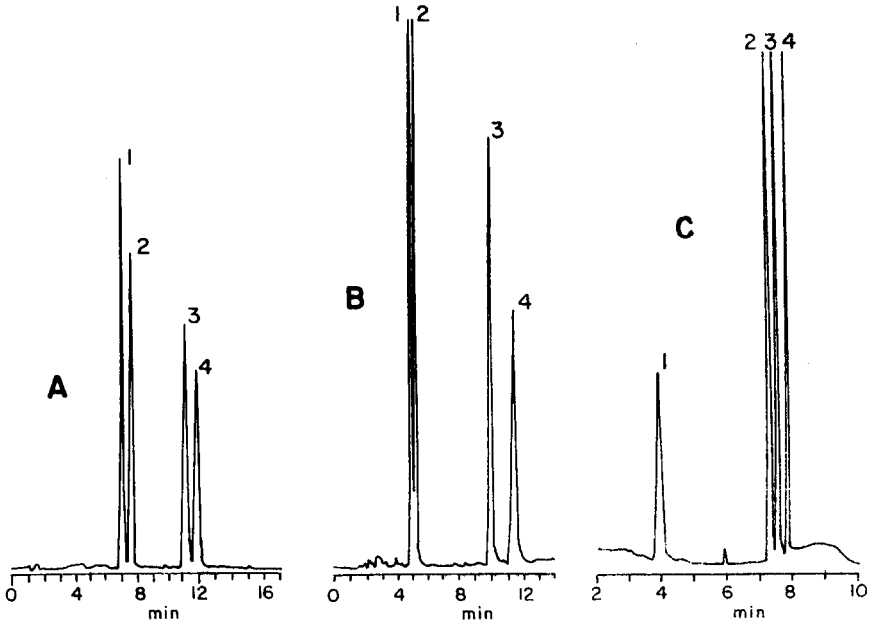


Fig. 1. (A and B) HPLC of phenoxy acids and phenoxy esters. (A) 1 = 2,4-D; 2 = MCPA; 3 = 2,4-DP; 4 = MCPP. (B) 1 = 2,4-D 2-butoxyethyl ester; 2 = MCPA 2-butoxyethyl ester; 3 = 2,4-D 2-ethylhexyl ester; 4 = MCPA 2-ethylhexyl ester. (C) GC of phenoxy esters. 1 = 2,4-D isobutyl ester; 2 = MCPA 2-butoxyethyl ester; 3 = 2,4-DP 2-butoxyethyl ester; 4 = 2,4-D 2-butoxyethyl ester.

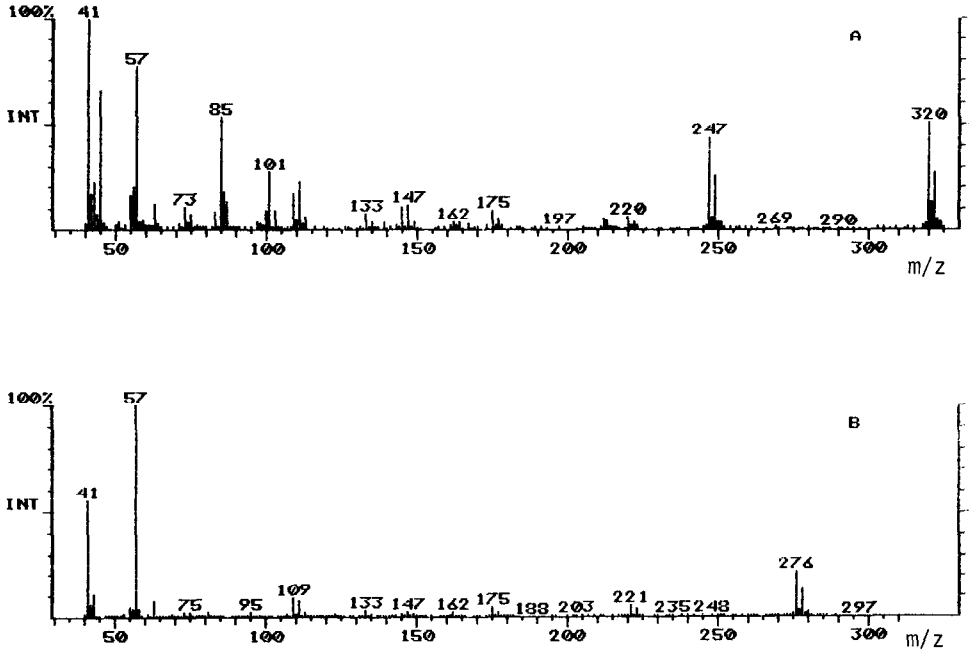


Fig. 2. Mass spectra of (A) 2,4-D 2-butoxyethyl ester and (B) 2,4-D isobutyl ester.

Several phenoxy esters were determined in technical materials and commercial formulations following the described procedures. Table III shows the results of the determination of these compounds as esters by HPLC and GC and after conversion to acids by HPLC. Good agreement between the HPLC and GC results was obtained in the ester determination. The equivalent values obtained by HPLC of the acids, based on the AOAC method [1], were also very close to those of the ester determination.

TABLE III

DETERMINATION OF PHENOXY ESTERS IN TECHNICAL MATERIALS AND COMMERCIAL FORMULATIONS

| Sample | | Active ingredient found ± S.D. (%)[a] | | |
|---|---|---|---|---|
| Acid | Ester | HPLC | | GC: ester |
| | | Acid | Ester | |
| 2,4-D | 2-Butoxyethyl | 31.14 ± 1.50 | 30.40 ± 0.83 | 29.20 ± 0.47 |
| 2,4-D | Isobutyl | 96.40 ± 1.30 | 97.30 ± 0.56 | 97.00 ± 0.48 |
| 2,4-D | Isobutyl | 37.00 ± 1.15 | 37.30 ± 0.85 | 37.70 ± 0.30 |
| 2,4-DP | 2-Butoxyethyl | 92.20 ± 2.74 | 92.00 ± 2.20 | 91.90 ± 1.30 |
| 2,4-DP | 2-Butoxyethyl | 31.00 ± 1.30 | 30.60 ± 1.06 | 29.80 ± 0.54 |
| MCPA | 2-Butoxyethyl | 23.20 ± 0.70 | 22.80 ± 0.50 | 22.60 ± 0.70 |
| MCPP | 2-Ethylhexyl | 93.30 ± 2.35 | 91.90 ± 1.45 | 91.90 ± 1.45 |

[a] Values are the means of five determinations ± standard deviation.

Nevertheless, the proposed methods determine the specific ester concentration instead of the acid equivalent concentration obtained with the AOAC method.

## CONCLUSION

The HPLC and GC methods proposed for the determination of phenoxy esters in formulations are simple, fast and reproducible. They allow the specific determination of esters with reliability and the results are in agreement with those obtained by the official method based on conversion to acids.

The HPLC method has the advantage that the phenoxy acids and the phenoxy esters can be analysed with the same column. On the other hand, the GC–ITD method allows the identification of the phenoxy esters from their mass spectra.

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## REFERENCES

1 K. Helrich (Editor), *Official Methods of Analysis of the Association of Official Analytical Chemists*, Arlington, VA, 1990, p. 184.
2 T. S Stevens, *Adv. Chromatogr.*, 19 (1981) 91.
3 J. Henriet, A. Martijn and H. H. Porlsen (Editors), *CIPAC Handbook*, CIPAC, Harpenden, 1985, p. 2069.
4 W. P. Cochrane, *J. Chromatogr. Sci.*, 17 (1979) 124.
5 E. A. Woolson and C. I. Harris, *Weeds*, 15 (1967) 168.
6 C. R. Sell and J. C. Maitlen, *J. Agric. Food Chem.*, 31 (1983) 572.
7 H. Siltanen and R. Mutanen, *Chromatographia*, 20 (1985) 685.
8 A. Noble, *Pestic. Sci.*, 23 (1988) 259.
9 J. P. Barrete and R. Payfer, *J. Assoc. Off. Agric. Chem.*, 47 (1964) 259.
10 A. Noble and D. J. Hamilton, *Pestic. Sci.*, 28 (1990) 203.
11 P. Jandera, L. Svoboda, J. Kubat, J. Schvantner and J. Churacek, *J. Chromatogr.*, 292 (1984) 71.
12 S. H. Hoke, E. E. Brueggemann, L. J. Baxter and T. Trybus, *J. Chromatogr.*, 357 (1986) 429.
13 C. H. Van Peteghem and A. M. Heyndrickx, *J. Agric. Food Chem.*, 24 (1976) 635.
14 C. S. Feung, R. H. Hamilton and R. O. Mumma, *J. Agric. Food Chem.*, 24 (1976) 1013.