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GAS–LIQUID CHROMATOGRAPHY AND GAS–LIQUID CHROMATOGRAPHY COMBINED WITH MASS SPECTROMETRY OF A BUTYL ESTER FORMULATION OF (2,4-DICHLOROPHENOXY)ACETIC ACID

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SUMMARY

A method is presented for the analysis of (2,4-dichlorophenoxy)acetic acid (2,4-D) in commercial formulations, together with concurrent detection and quantitation of related compounds. The method is based on direct gas–liquid chromatographic (GLC) analysis of the formulation to estimate the various esters present, and on an analysis of the total 2,4-D present involving a prior cleanup, alkaline hydrolysis, acidification and *n*-butylation steps. This procedure also quantitates any other related acids or phenols present. A step which would extract dioxin impurities is also included. The method was checked by the accepted acid–base estimation of 2,4-D content, GLC, and by subjecting radioactive-labelled herbicide to the above procedures.

INTRODUCTION

Comparatively few modern quantitative methods have been published concerning the amounts of the herbicide (2,4-dichlorophenoxy)acetic acid (2,4-D) and closely related chlorinated compounds present in commercial formulations. Some gas–liquid chromatographic (GLC) methods[1,2] are available but these are often not quantitative[3] and chemically related compounds are not analyzed. Some effort has also been devoted to the detection of possible chlorinated dioxins in 2,4-D (ref. 4) and 2,4,5-T (refs. 4,5) formulations. The usual methods of 2,4-D estimation for commercial formulations are by acid equivalent[3,6], total chlorine titrimetry[3,6], and by UV absorbance measurements[3]. Related compounds are also estimated along with the substrate, as these methods are non-specific.

Since herbicidal activity depends on the concentrations of the active ingredient of a formulation and as synergistic effects may occur when herbicides are mixed[7], it is essential that an accurate method of analysis be available for the quantitation of that component. In addition, such an analysis should also quantitate compounds which are closely related to the active herbicide. Workers should be cautioned against accepting the acid equivalent figure given by manufacturers of commercial formu-

lations since this may be subject to error, especially as few manufacturers include error ranges with the values of their acid equivalents, and so cause difficulties in the interpretation of data obtained from experiments where accurate dosages are required. Another problem relates to the environmental and metabolic degradation of herbicidal compounds such as 2,4-D, which is known to be photochemically dechlorinated[8], among other processes. If *o*- or *p*-chlorophenoxyacetic acids are found as degradation products of 2,4-D then a knowledge of the amounts (if any) present in a given formulation must be obtained before correct quantitation of the material obtained by degradation is possible, and before the existence of an environmental degradation cycle is confirmed.

We have developed a simple method for the determination of 2,4-D in ester formulations. This procedure allows concurrent detection and quantitation of related chlorinated compounds and the method is also applicable to ester formulations of other phenoxyacetic herbicides.

EXPERIMENTAL

Four 1-gallon cans of a commercially available "mixed butyl ester formulation of 2,4-D" (Chipman 2,4-D 128) were labelled A, B, C and D, respectively. The formulation was stated to contain "128 oz. of acid equivalent 2,4-D per imperial gallon of formulation" (*ca.* 0.8 g/ml).

The actual amount of total acid equivalent in each can was found by three independent methods:

(1) Quantitation of butyl esters by direct GLC measurement.

(2) Total free 2,4-D content by cleanup, then alkaline hydrolysis, followed by conversion of the free acid to *n*-butyl esters and subsequent GLC analysis.

(3) Quantitation of total free acid by the usual acid–base titration method before and after hydrolysis.

All solvents used were reagent grade, dried and distilled. Pure *n*-butyl and isobutyl esters of 2,4-D were prepared by the alcohol/BF_3 method[9]. The esters were distilled under reduced pressure, and known masses of esters were dissolved in hexane so that on injection into the gas chromatograph, the attenuation settings of the gas chromatograph used in the quantitations could be calibrated by means of area *versus* injected mass curves.

1. Quantitation by direct GLC measurement

One microliter of a solution of 0.1 ml of formulation in 100 ml of hexane was injected into a 1.83-m × 3-mm-I.D. GLC pyrex U-tube column and packed with 10% SE-30 on Chromosorb W (AW-DMCS). The injector, column, and ^{63}Ni electron capture detector (ECD) temperatures were 232, 187 and 222°, respectively. The flow-rate of the methane–argon (19:1) carrier gas was 27 ± 1 ml/min and the pulse interval was set at 15.

Each injection was repeated five times. Each peak was measured three times by planimetry, and the standard deviation calculated.

The *n*-butyl and isobutyl ester contents were estimated by reference to the calibration curves. The grand mean ester content and its associated standard deviation were then determined for each of the four cans.

2. Total free 2,4-D content by prior cleanup, alkaline hydrolysis, and conversion into n-butyl ester

A 0.1-ml aliquot of 2,4-D formulation was dissolved in 10 ml of aqueous saturated saline, and the solution extracted with acetonitrile (5 × 10 ml). This step separated the esters and other organic material from the surfactant. This does not occur if an acidified aqueous solution is used. The surfactant interferes with subsequent steps, and makes the final analysis impossible if not removed by this initial step. Other solvents such as hexane, benzene and *n*-butanol were much less efficient than acetonitrile.

Ten milliliters of 10% NaOH solution were added to the combined acetonitrile extract, and the acetonitrile was evaporated off under vacuum. The alkaline residue was refluxed for 20 min, cooled, and extracted once with 10 ml of hexane. This extraction removed any organic compounds which, after alkaline hydrolysis, were not water soluble. The GLC of this fraction showed that most of the "inert components" of the formulation were separated. This is the fraction which should also separate any chlorinated dioxins. None were detected by electron capture GLC.

The aqueous solution was then acidified with HCl to pH 2 (pHydrion paper) before extraction with benzene (3 × 10 ml). The benzene extracts contained the free carboxylic acids and phenols formed after acidification.

The combined benzene extract was concentrated to *ca.* 1 ml, 10 ml of *n*-butanol were added together with 3 ml of BF_3/*n*-butanol reagent (0.2 g/ml), and the mixture refluxed for 20 min. Ten milliliters of water were added after cooling. The *n*-butyl esters of the carboxylic acids, and any free phenols, were extracted with hexane (3 × 10 ml). The hexane extracts were combined and diluted to 100 ml.

GLC was then performed under the same conditions as described in the previous section. The procedure was repeated in triplicate for each sample, and the mean ester content as well as the associated standard deviation for each can were then calculated.

The efficiency of the entire procedure of extraction, hydrolysis, extraction after acidification, and butylation was 88 ± 2%, as shown by both radioactive assay and GLC. The initial acetonitrile extraction was *ca.* 99% efficient. The butylation as reported previously[9] was *ca.* 97% efficient. The hydrolysis and extraction steps were thus *ca.* 91% efficient.

Distilled and tap water subjected to the above procedure yielded no peaks at the retention time of the butyl esters of 2,4-D.

3. Quantitation of total free acid before and after hydrolysis by titration

Before hydrolysis. Esters, being neutral molecules, should exhibit a neutral pH in aqueous media. However, aqueous solutions of the formulations examined had an approximate pH of 3.5, suggesting partial hydrolysis of the ester to the free acid (which is not soluble alone in water), the presence of some other acidic component, *e.g.* a surfactant, or perhaps a combination of both. The following procedure was used to find the initial hydrogen ion concentration.

Ten milliliters of formulation were dissolved in 40 ml of water and immediately titrated with 0.0477 *N* NaOH solution using a pH meter to monitor the progress of the reaction. All titrations were done in triplicate.

After hydrolysis. A 0.1-ml aliquot of formulation was dissolved in 10 ml of

0.0477 *N* NaOH and then refluxed for 30 min. The excess NaOH was back-titrated with 0.0121 *N* HCl, using a pH meter. The amount of NaOH used to hydrolyze the ester could be found, as the original amount of NaOH was known. If the stoichiometry of the hydrolysis reaction is 1:1, then the mass of ester hydrolyzed can be calculated. All titrations were done in triplicate.

Identification of pesticidal components by GLC–MS

An 1.83-m × 3-mm-I.D. aluminum column containing 10% SE-30 on Chromosorb W (AW-DMCS) was used. The injector and flame ionization detector temperatures were both 250°. The flow-rate was 20 ml/min for the helium carrier, and 500 and 35 ml/min for the compressed air and hydrogen, respectively. The mass spectrometer was an MS-12 single-focussing instrument.

The column temperature was 195° for isothermal GLC. For temperature programming, the column was initially held at 80° for 12 min, then programmed at 12°/min to 190° until the ester peaks had eluted, and finally the temperature was raised to 290° until the baseline was steady again.

The obtained mass spectra were compared with those of authentic standards. Identification was considered positive if the compound had the same GC retention time and mass spectrum as the suspected compound.

RESULTS AND DISCUSSION

Table I shows the masses of *n*- and isobutyl esters and the resultant total acid equivalent of 2,4-D by direct GLC analysis. The total acid equivalents from the cleanup–hydrolysis–butylation procedure, and before and after alkaline hydrolysis as determined by acid–base titration, are also shown.

TABLE I

ANALYSIS OF MIXED BUTYL ESTER FORMULATION BY DIRECT GLC ANALYSIS, BUTYLATION* AND TITRATION

Can	*Mass of 2,4-D in 0.1-ml formulation (g) × 10^{-2}*					
	Direct GLC analysis			*Butylation acid equivalent*	*Titration acid equivalent*	
	n-Butyl ester	*Isobutyl ester*	*Total acid equivalent*		*Before hydrolysis*	*After hydrolysis*
A	5.5 ± 0.2	6.4 ± 0.2	9.4 ± 0.5	8.6 ± 0.4	0.045 ± 0.001	9.0 ± 0.5
B	6.0 ± 0.2	6.8 ± 0.3	10.7 ± 0.6	10.0 ± 0.4	0.048 ± 0.002	10.1 ± 0.5
C	6.2 ± 0.2	6.8 ± 0.2	10.3 ± 0.6	9.1 ± 0.5	0.046 ± 0.002	9.5 ± 0.3
D	9.4 ± 0.3	9.7 ± 0.3	15.3 ± 0.9	14.5 ± 0.7	0.050 ± 0.002	14.4 ± 0.8

* Corrected for inefficiencies.

The direct GLC analysis (Table I and Fig. 1a) shows that more isobutyl ester is present in the formulation than *n*-butyl ester. The *tert.*-butyl ester (peak 5 in Fig. 1a), as shown by its molecular weight and its retention time, was ignored in Table I as it represented only 1–2% of the total acid equivalent. However, it is quantitated in both procedures involving butylation and acid–base titration after

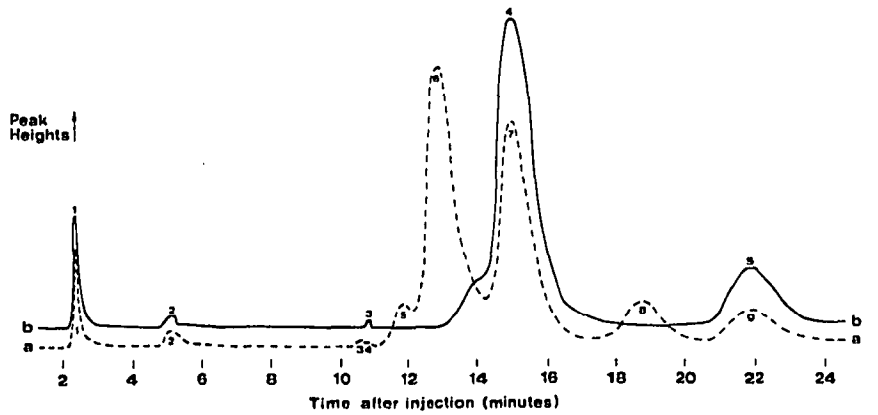

Fig. 1. Electron capture GLC analysis of commercial formulations of 2,4-D butyl esters. (a) Original formulation; (b) butylated formulation.

hydrolysis, as different esters of the same acid are reacted by the alkaline hydrolysis step to the same acid salt.

The total acid equivalents as found by direct GLC analysis, butylation and titration after hydrolysis, agree within 10%. The latter two methods give acid equivalents which are slightly lower than those found by direct GLC analysis. This is probably caused by a slight uncertainty in the actual GC curves for the formulated esters since they are not completely resolved under the conditions employed. However, the power of the two GC methods is shown by the fact that they both give answers within 10% of the usual titrimetric procedure. As Table I indicates, the standard deviations are *ca.* ± 5–8%. The 2,4-D levels in cans A and C are not significantly higher than their nominal content and can B has a slightly higher concentration than stated. However, can D has almost twice as much 2,4-D as expected. As all three independent methods agree, this content should be real. Thus, the results illustrate that the actual concentration of esters and the total acid equivalent of 2,4-D formulations should be checked before use, and taken into account in interpretation of results.

Table I also shows the amount of free acid in aqueous solution before alkaline hydrolysis. Again, can D contains a larger amount than the other cans. If the acidity was caused by free 2,4-D, it is only *ca.* 0.5% of the total acid equivalent. Free 2,4-D is likely to be present, as formulated butyl esters in water are almost completely hydrolyzed within two days.

Fig. 1a shows the composition of the formulation as detected by the ^{63}Ni ECD operated isothermally. Fig. 1b shows the formulation after cleanup, alkaline hydrolysis and butylation, run under the same GC conditions. The ^{63}Ni detector detected mostly chlorine-containing compounds, and the specificity of this detector is thus an advantage. This was borne out on temperature-programming GC using the flame ionization detector (FID), prior to MS, which showed the existence of at least 46 peaks, with peaks 1 and 2 of Fig. 1a being buried under the two peaks of the "inert" components of the formulation. The nature of the first peak in Fig. 1a was thus not investigated, but it has the same retention time as *n*-butyl chloroacetate. This is likely, as chloroacetic acid is used in the synthesis of 2,4-D. The peak is also present in

Fig. 1b, suggesting the component was originally a butyl ester or a phenol. However, on flame ionization GC–MS, it was too close to the solvent peak for identification.

Peak 2 in Fig. 1a is also present after the hydrolysis–butylation procedure (Fig. 1b) and had the approximate retention time of the isobutyl and *n*-butyl esters of phenoxyacetic acid.

The two small peaks (3 and 4) in Fig. 1a have the same retention times as the isobutyl and *n*-butyl esters of (*p*-chlorophenoxy)acetic acid. In addition, peak 4 is still present after hydrolysis–butylation (peak 3 of Fig. 1b) and its area is augmented by the area corresponding to peak 3 of Fig. 1a. Furthermore, temperature-progamming GC–MS analysis showed that peak 4 in both Figs. 1a and b has the same retention time and mass spectrum, with a molecular weight of 242 (one chlorine), as the *n*-butyl ester of (*p*-chlorophenoxy)acetic acid. This acid is a known depressor of 2,4-D activity[10].

Retention times, GC–MS and molecular weights (276 with two chlorines) indicate that peaks 5, 6 and 7 in Fig. 1a are the *tert.*-butyl, isobutyl and *n*-butyl esters of 2,4-D, respectively. Peaks 5 and 6 disappear on hydrolysis–butylation, but augment peak 7 (peak 4 in Fig. 1b) by their approximate areas. The shoulder on peak 4 (Fig. 1b) could not be resolved for unambiguous identification.

Peaks 8 and 9 in Fig. 1a could not be analyzed by flame ionization GC–MS because of their low concentration under isothermal conditions and because of high column bleed during temperature programming. However, after hydrolysis–butylation, peak 8 of Fig. 1a disappeared and augmented peak 9. The latter, as peak 5 in Fig. 1b, had a molecular weight of 310, with three chlorines, but the actual mass spectrum could not be obtained on temperature-programming GC–MS because of the high column bleed. However, the retention times of peaks 8 and 9 in Fig. 1a agreed with those given by the isobutyl and *n*-butyl esters, respectively, of (2,4,6-trichlorophenoxy)acetic acid. The presence of three chlorines explains the enhanced electron capture sensitivity and the poor flame ionization response. The content of this free acid was *ca.* 8×10^{-4} g/0.1 ml formulation, or *ca.* 1% of the total 2,4-D. However, this acid is a known depressor of 2,4-D activity[10], and, in addition, its mammalian toxicity is not documented.

Temperature-programming GC–MS using the FID showed the presence of at least 46 compounds. The chlorinated esters were eluted at the high-temperature end of the program, and most of the other peaks came before peaks 3 and 4 of Fig. 1a. Most of these compounds were hydrocarbons, as expected (hexanes and above). These were not investigated any further, since the emphasis was on chlorinated compounds.

No chlorinated benzo-*p*-dioxins were detected, either by GC–MS, MS alone, or in the hexane extract of the alkaline hydrolysis step where the dioxins might be expected to be extracted.

The sources of the various chlorinated species are probably the other phenols present in the 2,4-dichlorophenol substrate used in the synthesis of 2,4-D (ref. 11), *i.e.* phenol, *p*-chlorophenol and 2,4,6-trichlorophenol, the possible retention of unreacted chloroacetic acid used in the original synthesis of 2,4-D (ref. 12), and the mixture of alcohols used in the esterification.

The alkaline hydrolysis esterification method given here could also be used to reclaim the free phenoxyacetic acid quantitatively from obsolete forms of the herbi-

cide, *e.g.* the methyl or isopropyl esters of 2,4-D, or to reclaim expensive radiolabelled herbicides after use. In addition, the method involves a step to eliminate possible dioxin impurities.

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