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Journal of Chromatography A, 755 (1996) 245–250

JOURNAL OF
CHROMATOGRAPHY A

Determination of phenoxy acid herbicides in vegetation, utilising high-resolution gel permeation chromatographic clean-up and methylation with trimethylsilyldiazomethane prior to gas chromatographic analysis with mass-selective detection

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Received 22 April 1996; revised 2 July 1996; accepted 3 July 1996

Abstract

A method for the determination of phenoxy acid herbicides in vegetation samples is described. Macerated samples are extracted with acetone and following filtration and acidification, the herbicides are partitioned into dichloromethane. The herbicides are then cleaned-up using high-resolution gel permeation chromatography before undergoing rapid and efficient methylation using trimethylsilyldiazomethane. The resultant methyl esters are then selectively and sensitively analyzed by gas chromatography with mass selective detection. Silanized glassware was used throughout. The method has been applied to grass samples spiked with four phenoxy acid herbicides; 2,4-D, dichlorprop, MCPA and mecoprop. Recoveries around 100% and reproducibilities of 5% ($n=4$) were achieved at two spiking levels, ~ 700 and ~ 140 $\mu\text{g}/\text{kg}$.

Keywords: Environmental analysis; Derivatization, GC; Pesticides; Phenoxy acid herbicides; Trimethylsilyldiazomethane

1. Introduction

Phenoxy acids were first introduced as herbicides in the late 1940s. They have since found widespread usage in the post-emergence control of annual and perennial broad leaved weeds in cereals and grasses. Functioning as synthetic plant growth regulators (PGRs) these herbicides accumulate in the roots and stems [1] of the plant, i.e., the growing areas. Trace levels of 2,4-D, for example, will stimulate elongation growth, as does indoleacetic acid (IAA), one of the plants' natural PGRs. Higher concentrations are required to produce the inhibitory effects associated with these herbicides.

Spray drift is a problem associated with the widespread use and availability of phenoxy acid herbicides and pesticides in general. In addition to the potential health problems, this can result in herbicides being deposited onto susceptible crops, causing extensive damage [2]. For monitoring and enforcement purposes, a reliable method for determining phenoxy acids in vegetation is clearly required. The complexity of the matrices involved combined with the low levels of contamination and the particular physical and chemical properties of the phenoxy acid herbicides make their determination in environmental samples difficult. Grass samples have been chosen for the purposes of this work, being a common matrix exposed to spray drift.

Published methods for the extraction and clean-up

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of phenoxy acids have employed solid-phase extraction for water samples [3] and Florisil column clean-up following solvent extraction and derivatization with pentafluorobenzyl bromide for vegetation samples [4]. The poor recoveries (<60%) experienced in our laboratory when using the latter method led us to investigate alternative procedures.

In our laboratory, a multi-residue procedure employing gel permeation chromatography (GPC) clean-up, has been successfully applied to the determination of organochlorine, organophosphorus, organonitrogen and synthetic pyrethroid pesticides in vegetation samples. Samples are extracted with acetone, liquid–liquid partitioned with dichloromethane (DCM) and cleaned-up with GPC prior to gas chromatography (GC) or high-performance liquid chromatography (HPLC). We now report adaptations to the procedure enabling the extraction and clean-up of phenoxy acid herbicides. The use of silanized glassware and a lowered pH at the liquid–liquid partition stage have resulted in good recoveries from grass samples.

Due to low volatility and poor thermal stability, a number of the phenoxy acids cannot be determined directly by GC and require prior derivatization. Several derivatization techniques have been reported [5] including: esterification with pentafluorobenzyl bromide and electron-capture detection [4,6], esterification using either methyl or ethyl chloroformate and flame ionization detection [7], methylation with diazomethane and mass-selective detection (MS) [3,8] and silylation with a cyano-containing group and nitrogen–phosphorus detection [9–12]. We have chosen to prepare the methyl ester derivatives using trimethylsilyldiazomethane, a less hazardous but equally effective substitute for diazomethane [13], which has been shown to react quickly and cleanly at room temperature [14]. GC–MS analysis was employed, producing more sensitive and selective determinations from cleaned-up grass extracts.

2. Experimental

2.1. Chemicals and solutions

2,4-D, dichlorprop, MCPA and mecoprop (see Fig. 1) were supplied as the free acids and methyl esters (Promochem, Welwyn Garden City, UK) with cer-

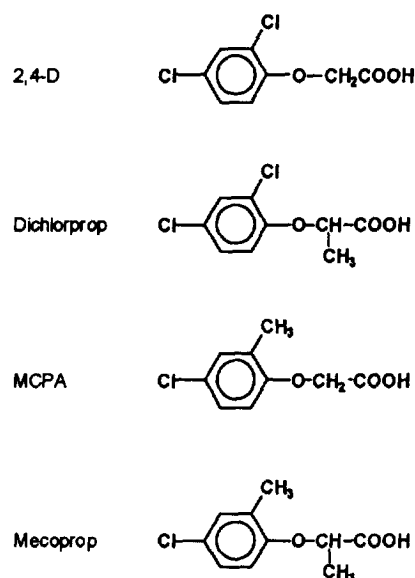


Fig. 1. Chemical structures of the four phenoxy acid herbicides used in this work.

tified purities ranging from 99.0 to 99.7%. Five working standards in the range 0.1–1.0 $\mu\text{g}/\text{ml}$ were prepared in acetone from a 50 $\mu\text{g}/\text{ml}$ methyl ester stock solution. A 250 $\mu\text{g}/\text{ml}$ solution of the free acids in acetone was prepared as a spiking standard. All standard and stock solutions were stored in amber bottles at 2–8°C.

The trimethylsilyldiazomethane (Aldrich, Gillingham, UK) for the derivatization was supplied and used as 2.0 *M* solution in hexanes. The dichlorodimethylsilane (99%, Aldrich) for the silanization of glassware was diluted with cyclohexane to give a 5% solution, and the hydrochloric acid, 37% solution (99.999%, Aldrich) was diluted with LC-grade water to give a 1.0 *M* solution. The anhydrous sodium sulphate (Fisher Scientific UK, Loughborough, UK) was analytical-reagent grade and was heated to $400 \pm 25^\circ\text{C}$ for 4 h. All solvents were Distol grade (Fisher). The DCM was vacuum filtered through a Whatman nylon filter (47 mm diameter, 45 μm pore size) immediately prior to use on GPC.

2.2. Instrumentation and apparatus

All glassware was deactivated by rinsing thoroughly with a 5% solution of dichlorodimethylsilane

in cyclohexane. The glassware was then rinsed three times in cyclohexane and washed (end-capped) with methanol.

The GPC system comprising a Waters 510 HPLC pump and 717 plus autosampler attached to two Waters Envirogel GPC clean-up columns, a guard column (150×19 mm) and a main clean-up column (300×19 mm). A Perkin-Elmer LC 135 diode-array detector set at 255 nm and a Waters fraction collector were used. The flow-rate was set at 5.0 ml/min and the mobile phase was DCM at ambient temperature.

The GC-MS system consisted of a Hewlett-Packard 5890 series II gas chromatograph fitted with a Hewlett-Packard HP-5 MS column (cross-linked 5% phenyl silicone, 30 m×2.5 mm×2.5 μm film thickness), a Hewlett-Packard 5972 Series mass-selective detector with Hewlett-Packard G1034C MS ChemStation software. The injection (splitless) and transfer line temperatures were 250 and 280°C respectively and the oven temperature programme was 60°C for 1 min, ramping at 15°C/min to 295°C for 5 min. Helium (>99.996%) was used as the carrier gas and electronic pressure control in constant flow mode delivered 0.98 ml/min. Selected ion monitoring (SIM) data was collected between 9.0 and 17.0 min monitoring ions with *m/z* values of 228 and 230 for mecoprop, 214 and 216 for MCPA, 248 and 250 for dichlorprop and 234 and 236 for 2,4-D. The first ion listed for each analyte was for quantification and the second for confirmation.

A TurboVap II Concentration Workstation (Zymark, Warrington, UK) was set for an end-point of 0.5 ml with a bath temperature of 40°C for the concentration of samples under nitrogen.

2.3. Procedures

2.3.1. Spiking vegetation

The grass was spiked at two levels, approximately 5 μg and 25 μg of each herbicide per 35 g of vegetation (i.e., at ~140 μg/kg and ~710 μg/kg, respectively). This was performed by injecting 20 μl and 100 μl of the 250 μg/ml free acid stock solution onto blank samples of the plant material. The spiked samples were then covered and left overnight in a refrigerator at between 2–8°C. Four replicate spiked samples were prepared at each level.

2.3.2. Extraction

Each spiked grass sample was homogenized for 3 min with acetone (100 ml) using a blender. The blended grass sample was filtered under vacuum through a porosity 1 sintered glass funnel into a 500-ml Büchner flask, the filter cake being washed twice with acetone (20 ml). The filtrate was transferred to a 500-ml separating funnel where DCM (100 ml) was added and the flask shaken vigorously for 30 s. Once the two layers had separated the pH of the aqueous upper layer was adjusted to below pH 2 with dropwise addition of hydrochloric acid (1.0 M). The contents of the flask were reshaken for 30 s and the layers again allowed to separate. The lower organic layer was poured into a 600-ml beaker. This extraction was repeated twice more with DCM (50 ml). The combined organic phase was dried by stirring with anhydrous sodium sulphate (20 g) and then filtered under vacuum through a porosity 4 glass funnel into a 500-ml Büchner flask. The filtrate was transferred to a 500-ml round-bottomed flask and concentrated just down to dryness using a rotary evaporator. The residue was transferred to a 5-ml volumetric flask and made up to the mark using DCM. The crude extract was filtered through a syringe filter (0.45-μm pore size) attached to a disposable, plastic 5-ml syringe. About 4 ml of the filtrate was collected in an amber glass vial ready for GPC clean-up.

2.3.3. GPC clean-up

A 1-ml volume of the filtered crude extract was injected onto the GPC columns using the conditions mentioned previously. The eluent was collected between 13 and 19 min in fraction collector tubes, the retention times having been previously determined by running a free acid standard. The fractions collected were then transferred to a TurboVap tube and evaporated down to 0.5 ml before being taken more carefully to near dryness on a TurboVap Concentration Workstation.

2.3.4. Derivatization

The residue was dissolved in 2 ml of a methanol-toluene (20:80) mixture. A 25-μl volume of trimethylsilyldiazomethane (2.0 M) was added to the solution and the tube was covered using laboratory sealing film and sonicated for 20 min. The reaction mixture was then concentrated down to 0.5 ml before

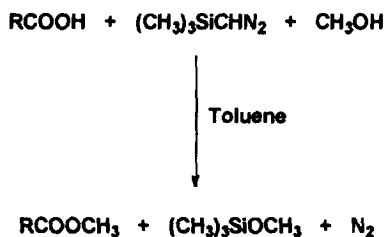


Fig. 2. Reaction scheme showing an acid group reacting with trimethylsilyldiazomethane to give the methyl ester and side products.

being taken to near dryness on a TurboVap evaporator (see Fig. 2 for reaction scheme). The residue was transferred to a 2-ml (for low spiking level) or a 10-ml (for high spiking level) volumetric flask and made up to the mark with acetone and then transferred to a GC vial for analysis by GC-MS.

3. Results and discussion

Fig. 3, panel 1 shows that the baseline resolution of all four analyte peaks was achieved for the standard solutions, with all of the four phenoxy acid esters eluting between 9 and 11 min. Fig. 3, panels 2 and 3 show typical chromatograms from grass samples spiked at the low and high levels respectively with the four phenoxy acids following extraction, clean-up and derivatization. The methyl ester peaks are clearly resolved from any co-extractants. Analysis of the blank sample revealed no trace (i.e., below limit of detection) of the herbicides except for 2,4-D

(found to have been applied previously) which was quantified at 18 $\mu\text{g}/\text{kg}$.

The results of the recovery experiments are shown in Table 1 (2,4-D has been corrected for the contamination found in the blank). There was little variation in the recoveries between the higher ($\sim 710 \mu\text{g}/\text{kg}$) and the lower ($\sim 140 \mu\text{g}/\text{kg}$) spiking levels and all four pesticides were recovered satisfactorily with recovery values of between 98.0 and 107.0%. The mean results and standard deviations (S.D.) are calculated from four repeat experiments at each spiking level.

These results show a marked improvement over the use of the standard in-house multi-residue method, where the liquid-liquid partitioning was carried out at neutral pH and untreated glassware was used. Recoveries of around 50% were typical for the phenoxy acid herbicides under the standard method conditions. The improved recoveries can thus be credited to:

- (1) The deactivation of all glassware, which prevents adsorption of the acids onto the active sites of the glass.
- (2) A lowering of the pH at the liquid-liquid partitioning step, which ensures that the analytes are present as free acids, these being more soluble in the organic phase (DCM-acetone) at the partitioning stage than their corresponding carboxylate ions.

The low limits of detection (typically 2–9 $\mu\text{g}/\text{kg}$) achieved with the GC-MS analysis operated in SIM mode are sufficiently sensitive for the determination of these pesticides in samples exposed to spray drift. This has been illustrated by the detection of 2,4-D

Table 1
Mean recoveries of phenoxy acids from spiked samples of vegetation

Phenoxy acids	Spiking levels ($\mu\text{g}/\text{kg}$)	% Recovery (mean \pm S.D.)	Limit of detection ($\mu\text{g}/\text{kg}$)
2,4-D	143	105.6 \pm 4.8	9
	714	104.2 \pm 6.7	
Dichlorprop	182	98.0 \pm 5.9	9
	909	102.1 \pm 5.0	
MCPA	185	107.0 \pm 5.9	4
	923	105.6 \pm 5.3	
Mecoprop	186	103.0 \pm 6.3	2
	929	101.0 \pm 5.5	

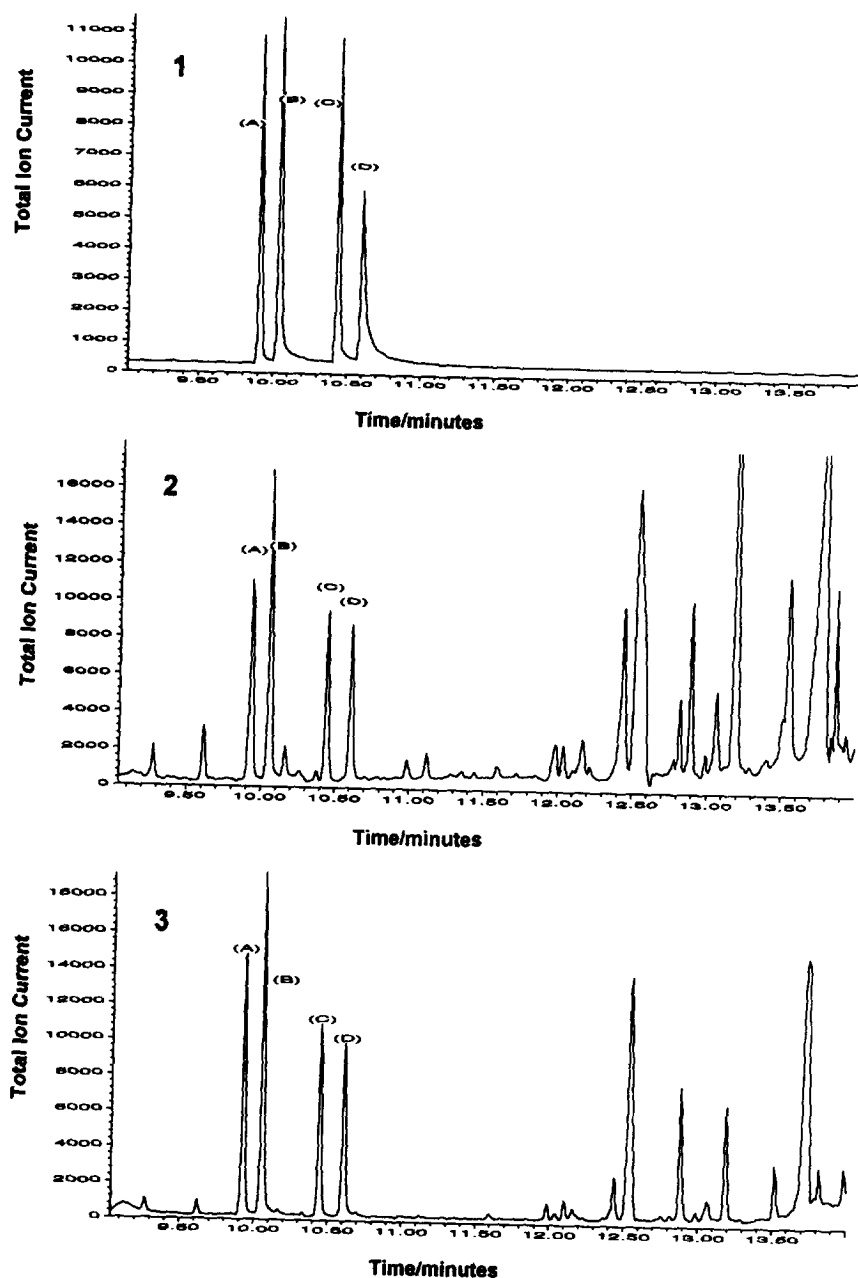


Fig. 3. Examples of total ion currents for the GC-MS-SIM analysis for phenoxy acid methyl esters showing (A) mecoprop, (B) MCPA, (C) dichlorprop and (D) 2,4-D. Chromatogram 1 shows the 0.5 ppm standard solution, chromatogram 2 shows a low level spiked vegetation sample and chromatogram 3 shows a high level spiked vegetation sample.

residues at $18 \mu\text{g}/\text{kg}$ in the grass cuttings collected for this study. The derivatization reaction utilising trimethylsilyldiazomethane with methanol provides a less hazardous method for preparing the phenoxy

acid methyl esters under mild conditions than with diazomethane. Trimethylsilyldiazomethane is commercially available obviating the need to synthesize diazomethane. The good recoveries achieved from

grass samples using a short reaction time, suggest that the reaction is efficient, quantitative and that it is not influenced by the presence of other compounds that may have been co-extracted from the vegetation matrix.

The procedure offers significant benefits over other reported methods in that we have applied a variant of an established multi-residue screening procedure, thus preventing the need for a separate specific method for phenoxy acids.

4. Conclusions

The adapted multi-residue method described in this paper provides a simple route to the determination of four phenoxy acids in vegetation samples. Good reproducible recoveries have been achieved using the modified extraction procedure with GPC clean-up. Subsequent GC–MS–SIM analysis of the phenoxy acid methyl esters, prepared under mild reaction conditions using trimethylsilyldiazomethane, enables sensitive and selective determination. Details of field evaluations and results on lower-level spiking experiments will be investigated in future work.

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