Adaptation and application of a multi-residue method for the determination of a range of pesticides, including phenoxy acid herbicides in vegetation, ased on high-resolution gel permeation chromatographic clean-up and gas chromatographic analysis with mass-selective detection

Paul D. Johnson, Duncan A. Rimmer*, Richard H. Brown

Health and Safety Laboratory, Broad Lane, Sheffield, S3 HQ, UK

Abstract

A multi-residue method to determine pesticides in vegetation samples is described. Blended samples were extracted with acetone and the pesticides partitioned into dichloromethane prior to being cleaned-up using high-resolution gel permeation chromatography. Investigations were made into the effects of altering the pH of the aqueous phase during partitioning. Rapid and efficient methylation of the phenoxy acids present was achieved using trimethylsilyldiazomethane. The resultant solutions were then selectively and sensitively analysed by gas chromatography with mass-selective detection. The variation in results using matrix matched and non-matrix matched calibration standards was also explored. The method has been applied to grass samples spiked with pesticides from the following groups, phenoxy acid, organophosphorus, triazine, organochlorine, phenyl urea, chloroacetanilide and pyrethroid. Recoveries around 80–100% and reproducibilities of 2–4% ($n=4$) were typically achieved at a spiking level ~140 μg kg^{-1}

Keywords: Sample preparation; Environmental analysis; Pesticides; Phenoxy acid herbicides

1. Introduction

Spray drift is a problem associated with the widespread use of pesticides. In addition to the potential health problems, this can result in herbicides being deposited onto susceptible crops, causing extensive damage [1]. For monitoring and enforcement purposes, a reliable method for determining pesticides in vegetation is clearly required. The complexity of the matrices involved combined with the low levels of pesticide contamination make their determination in environmental samples difficult. This is acutely so for the phenoxy acid herbicides due to their particular physical and chemical properties.

Published methods for the determination of pesticides in vegetation samples have employed gel permeation chromatography (GPC) [2,3], solid-phase extraction (SPE) [4–8] and supercritical fluid extraction (SFE) [9,10] for sample clean-up prior to chromatographic analysis. Of the three techniques, GPC appears to be more widely applicable for a multi-residue method, allowing the clean-up of both

*Corresponding author.

polar and non-polar pesticides [11] within the same injection on a fully automated system.

In our laboratory, a multi-residue procedure has been successfully applied to the determination of organochlorine, organophosphorus, organonitrogen and synthetic pyrethroid pesticides in vegetation samples [12]. Samples, usually grass found in or around the area where spray drift may have occurred, are extracted with acetone, liquid–liquid partitioned with dichloromethane (DCM) and cleaned-up with high-resolution GPC before analysis by gas chromatography with mass-selective detection (GC–MS). We have previously reported a variant of this multi-residue method specifically for the determination of phenoxy acid herbicides [13], employing pH adjustments at the liquid–liquid partitioning step and the use of trimethylsilyldiazomethane [14–16], for the methylation of phenoxy acids prior to GC–MS analysis.

The aims of this study have been to investigate how pH adjustments to the aqueous phase at the partitioning stage effect the extraction efficiencies of phenoxy acid herbicides, as well as a range of other classes of pesticides and to illustrate the effectiveness of calibrating with matrix matched standards [17,18]. It was also intended to show that the optimized extraction procedure is reliable in the determination of 26 representative pesticides within the same multi-residue method.

2. Experimental

2.1. Chemicals and solutions

All pesticide mixes were supplied (Qm$_x$ Labs., Great Yeldham, Halsted, UK) at a concentration of 10 μg ml^{-1} or 200 μg ml^{-1} with a certified tolerance of ±0.5%. Chlorpyriphos, α-endosulfon, lindane, 4-(4-chloro-2-methylphenoxy)butanoic acid (MCPB), cis-permethrin, trans-permethrin and (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T) were obtained individually (Promochem, Welwyn Garden City, UK) with certified purities ranging from 99.0 to 99.7%, these were prepared gravimetrically as 100 μg ml^{-1} solutions in cyclohexane or ethyl acetate. The pesticides involved in this study are from the following chemical groups; phenoxy acid, phenyl

urea, triazine, organophosphorus, organochlorine, chloroacetanilide and pyrethroid and are listed in Table 1. The phenoxy acids were supplied as both free acids (for spiking) and the methyl ester (for calibration). Two sets of five working standards in the range 0.2–0.6 μg ml^{-1}, one matrix matched and the other non-matrix matched, were prepared gravimetrically in acetone from a 5 μg ml^{-1} stock solution. Matrix matched standards were prepared by the addition of 0.5 ml of double concentration grass extract to the appropriate volume of stock solution in a 1.0 ml volumetric flask, this then being made up to the mark with cyclohexane-ethyl acetate (50:50, v/v). All standards 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 a 2.0 M solution in hexane. The dichlorodimethylsilane (99%, Aldrich) for the silanization of glassware was diluted with cyclohexane to give a 5% solution and the hydrochloric acid, (37%, 99.999%, Aldrich) was diluted with LC grade water to give a 1.0 M solution. The sodium hydroxide (Aldrich) was supplied as a 1.0 M volumetric standard. The anhydrous sodium sulphate (Fisher, Loughborough, UK) was analytical-reagent grade and was heated to 400±25°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 the GPC system.

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 comprised of a Waters 510 HPLC pump and 717plus 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^{-1} and the mobile phase was DCM at ambient temperature.

The GC–MS system consisted of a Hewlett-Pac-

Table 1
Peak identification and SIM conditions for GC–MS analyses

Peak no.	Pesticides in standards	SIM group	SIM ion	Retention time (min)	Limit of detection[a] (μg kg^{-1})
1	Mecoprop methyl ester	1	228	9.99	1
2	MCPA methyl ester		214	10.17	1
3	Dichlorprop methyl ester		162	10.72	4
4	2,4-D methyl ester		199	10.99	1
5	Demeton	2	88	12.34	18
6	Simazine		201	12.55	6
7	Atrazine		200	12.74	2
8	Lindane	3	217	13.11	6
9	Terbutylazine		214	13.27	2
10	MCPB methyl ester		101	13.39	3
11	2,4,5-T methyl ester		268	13.43	3
12	Diazinon		304	13.73	1
13	Disulfoton		88	13.89	8
14	Sebuthylazine	4	200	14.51	2
15	Metobromuron		258	14.73	14
16	Parathion methyl		263	15.55	5
17	Malathion	5	125	17.12	27
18	Chlorpyriphos		314	17.49	2
19	Cyanazine[a]		225	17.54	11
20	Parathion ethyl		291	17.52	1
21	Metazachlor	6	209	18.61	14
22	α-Endosulfon		241	19.58	1
23	Ethion	7	231	20.99	2
24	Azinphos methyl		160	22.62	30
25	cis-Permethrin		183	23.31	4
26	trans-Permethrin		183	23.39	4

[a]Determined using a matrix matched standard.

kard 5890 series II gas chromatograph fitted with a Hewlett-Packard HP-5 MS column (cross-linked 5% phenylsilicone, 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^{-1} to 180°C and holding for 4 min, ramping at 5°C min^{-1} to 205°C then 20°C min^{-1} to 295°C holding for 5 min, total run time is 27.5 min. Helium (<99.996%) was used as the carrier gas and electronic pressure control in constant-flow mode delivered 0.98 ml min^{-1}. Selected ion monitoring (SIM) data was collected between 9.0 and 25.0 min (see Table 1).

A TurboVap II Concentration Workstation (Zymark, Warrington, UK) was set for a 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

Each grass sample was spiked at approximately 5 μg of each herbicide per 35 g of vegetation (i.e. at ~140 μg kg^{-1}). This was performed by gravimetrically weighing ~0.5 ml of the 10 μg ml^{-1} pesticide mix, ~25 ml of the 200-μg ml^{-1} pesticide mix and ~50 μl of the individual 100-μg ml^{-1} pesticide solutions into a silanized glass vial, this was then transferred onto blank samples of the plant material. The spiked samples were then covered and left overnight in a refrigerator at 2–8°C.

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. The five separate extraction procedures (A–E) shown in Fig. 1 were then followed. The pH of the aqueous upper layer was lowered to 2 with dropwise addition of 1 M HCl and raised to 10 by dropwise addition of 1 M NaOH. 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 down to below 1 ml using a rotary evaporator. The residue

Fig. 1. Schematic representation of modified extraction procedures.

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

Filtered crude extract (1 ml) was injected onto the GPC columns using the conditions mentioned previously. The blank grass extract (2 ml) was injected, giving a double concentration grass extract for preparation of the matrix matched standards. The eluent was collected between 11.75 and 21.75 min in silanized fraction collector tubes. The retention time of each pesticide having been previously determined by running a standard of each pesticide through the GPC, with lindane each 1-min fraction was collected and determined by GC–MS as it does not absorb at 255 nm. 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 portion of 2 M trimethylsilyldiazomethane was added to the solution and the tube was covered using laboratory sealing film and sonicated for 30 min. The reaction mixture was then concentrated down to 0.5 ml before being taken to near dryness on a TurboVap evaporator. The residue was transferred to a 2 ml volumetric flask and made up to the mark with cyclohexane-ethyl acetate (50:50) and then transferred to a GC vial for analysis by GC–MS.

3. Results and discussion

Fig. 2 shows the total ion chromatogram (TIC) from a GC–MS scan of the pesticide stock mix (~5 μg ml^{-1}). Not all peaks were resolved in the mix, but when the ions listed in Table {r}1 were monitored for as part of a SIM method, the unresolved peaks could be individually integrated enabling successful quantitation. Fig. 3A shows the SIM-TIC for the highest matrix matched standard which appears very
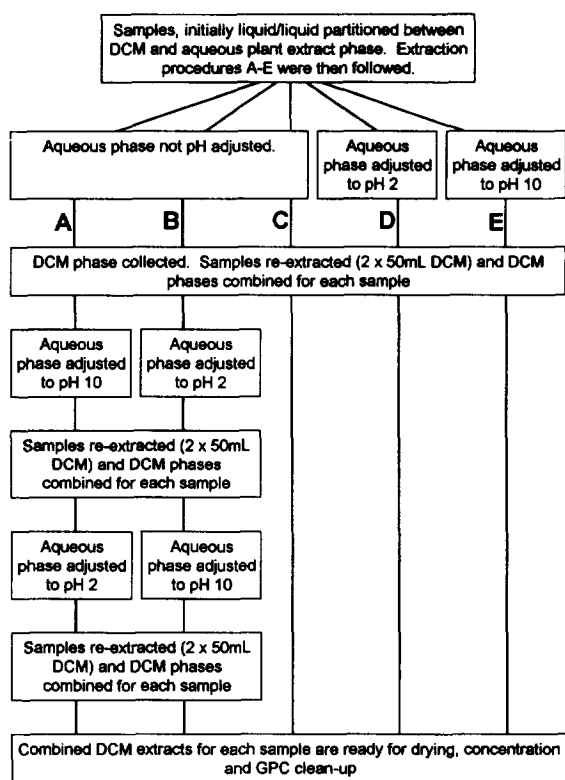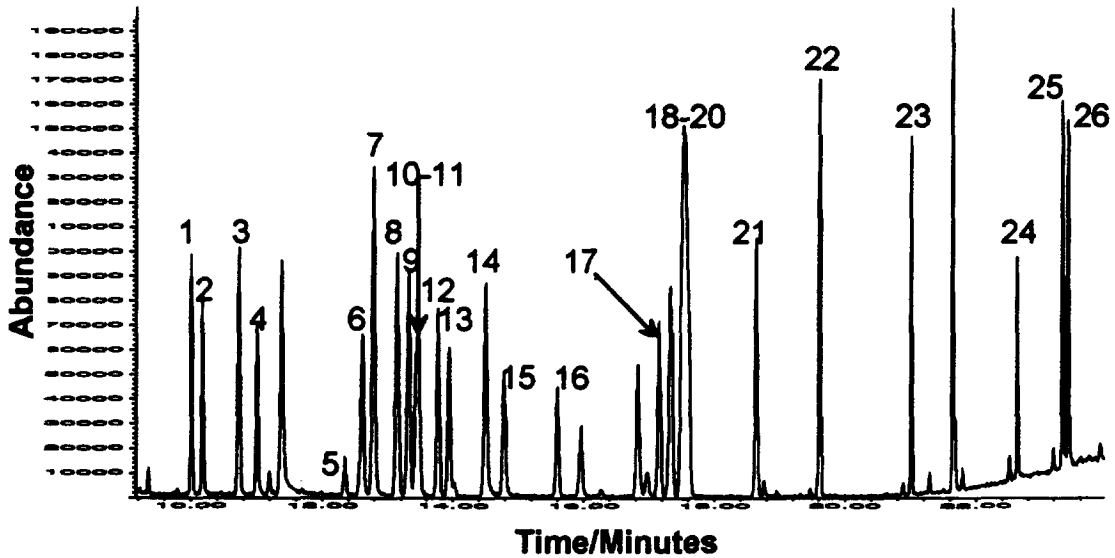
Fig. 2. GC–MS chromatogram of the pesticide mixture.

complicated. Fig. 3B shows the same chromatogram but with only six ions selected, the individual analyte peaks are clearly visible and this is also the case for co-eluting analytes. Analysis of the blank sample revealed no trace (i.e. below limit of detection) of the pesticides except for 2,4-D (found to have been applied previously) which was quantified at 18 μg kg^{-1}.

All calibration graphs were linear over the standard range i.e. 0.2–0.6 μg ml^{-1} (linear correlation coefficients were in the range of 0.992–1.000). The use of matrix matched standards improved the peak shapes for most of the pesticides, the effects of which produced improved linearities and intercepts closer to the origin, both improving the accuracy of the calibration. These effects were more noticeable with polar pesticides such as the triazines (cymazine, simazine etc.) than with the less polar ones (e.g. the phenoxy acid methyl esters).

The limits of detection (LOD) were determined from three times the signal-to-noise ratio (S/N) calculated from a macro in the HP G1034C MS CHEMSTATION software for the lowest matrix matched standard. The LODs, which are shown on Table 1, range from 1–30 μg kg^{-1} are acceptable for the residue analysis required to determine the extent of spray drift.

The results of the recovery experiments are shown in Table 2 (non-matrix matched standards) and Table 3 (matrix matched standards). The tables show the results for the different partitioning methods (A–E) as well as giving the mean and standard deviation from replicate spiked samples which were extracted and partitioned using method A.

The non-matrix matched calibrated results show recoveries considerably higher than the 100% expected for certain pesticides (e.g. parathion ethyl 141–187%), but not others (e.g. the phenoxy acid methyl esters). However, the same samples analysed against the matrix matched calibration standards show more sensible recoveries in the 70–110% range. The recoveries of certain pesticides (e.g. phenoxy acid methyl esters, diazinon, chlorpyriphos and permethrin) do not appear to differ greatly between the use of matrix matched and non-matrix matched calibration. It is thought that such pesticides are less polar and hence are less susceptible to adsorption by the active sites in the injection liner. Overall the results indicate that the use of matrix matched calibration standards should be adopted for a multi-residue screening method.

The results from the different partitioning procedures show that a neutral and preferably a low pH extraction should be included if screening is to include the phenoxy acid herbicides. The recoveries of most of the pesticides appear to be marginally
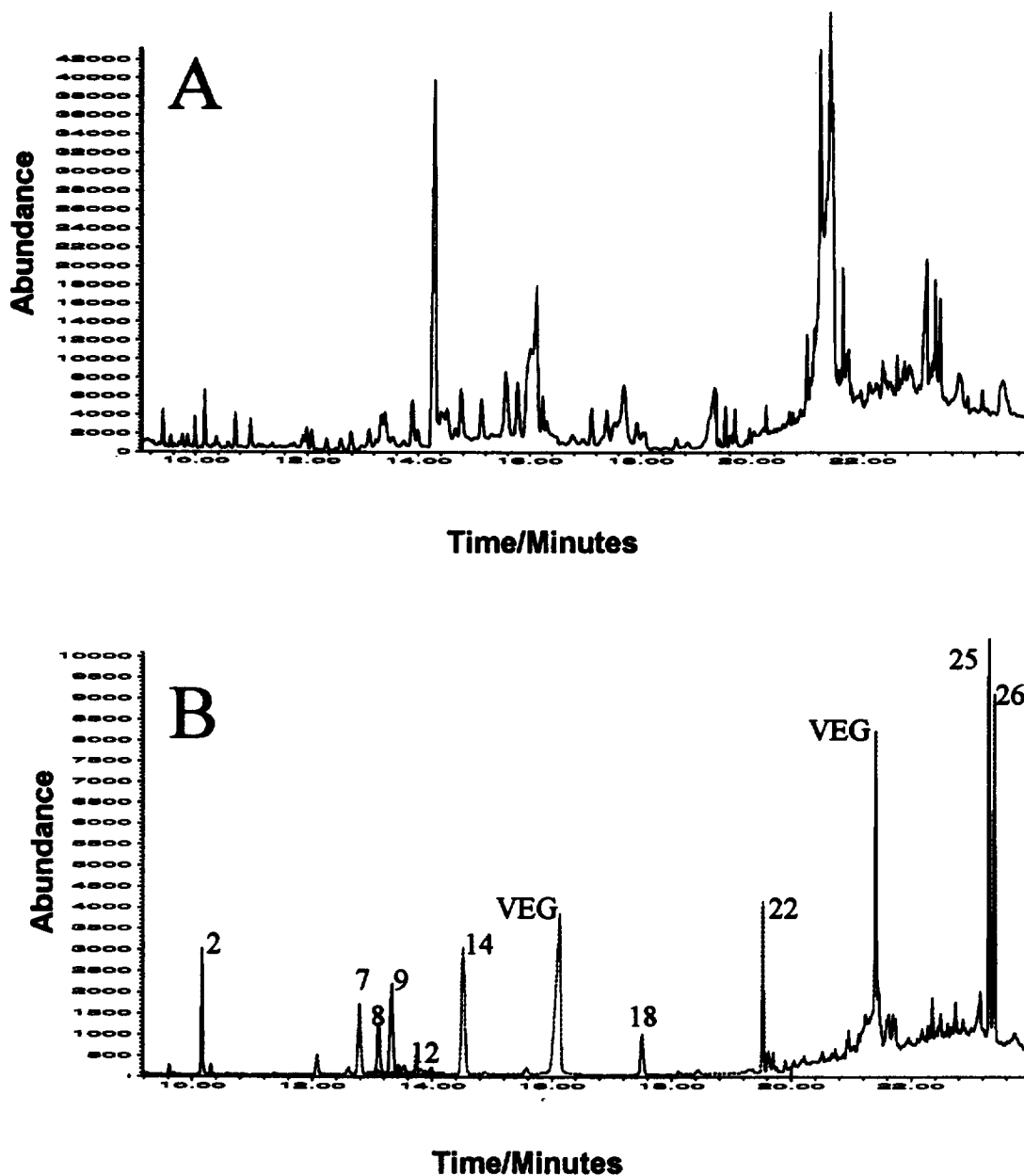
Fig. 3. Examples of GC-MS chromatograms for pesticide residues in vegetation, showing the effectiveness of SIM in resolving the analytes from the matrix. Chromatogram A shows the SIM-TIC for the 600 ng ml^{-1} matrix matched standard. Chromatogram B shows the same GC-MS chromatogram after several single ions have been selected. Peak identification numbers are as used in Table 1.

greater if the samples are partition at neutral, high and then low pH values 2,4,5-T appears to be the only pesticide which gives a noticeably low re-

covery, although this may be due to a low reaction yield at the derivatization step.

The derivatization reaction utilising tri-

Table 2
Percentage recoveries of pesticides from spiked samples of vegetation usingnon-matrix matched calibration standards

Peak	Pesticides	Recovery from the five modified partitioning methods (A–E) studied (%)				
		A[a]	B	C	D	E
1	Mecoprop	85±1.2	87	70	93	0
2	MCPA	78±5.0	71	59	77	0
3	Dichlorprop	87±11.4	94	79	96	0
4	2,4-D[b]	81±7.3	116	89	117	0
5	Demeton	130±17.4	105	101	88	67
6	Simazine	115±3.3	131	121	121	117
7	Atrazine	116±3.3	127	116	118	114
8	Lindane	59±1.0	67	62	66	69
9	Terbutylazine	112±4.5	125	117	118	115
10	MCPB	107±2.1	150	118	132	0
11	2,4,5-T	18±1.3	24	17	24	0
12	Diazinon	96±3.0	101	92	101	91
13	Disulfoton	103±11.0	81	74	71	77
14	Sebuthylazine	115±2.6	Not analysed			
15	Metobromuron	91±5.5	132	137	146	132
16	Parathion methyl	139±4.7	192	178	183	162
17	Malathion	119±3.9	124	125	123	120
18	Chlorpyriphos	101±4.2	77	91	79	77
19	Cyanazine	139±5.4	135	123	132	118
20	Parathion ethyl	141±5.7	187	174	176	150
21	Metazachlor	116±4.1	149	136	145	136
22	α-Endosulfon	72±3.8	85	82	80	87
23	Ethion	102±2.1	118	126	116	125
24	Azinphos methyl	71±5.1	93	117	105	137
25	cis-Permethrin	80±2.8	96	97	91	108
26	trans-Permethrin	85±5.0	91	93	98	99

[a]Mean of four replicate analyses ($n=4$)±one standard deviation.
[b]Blank sample was found to be contaminated with 2,4-D.

methyldiazomethane, offers significant benefits over other reported methods in that a safe and efficient reaction is employed under mild conditions. Recoveries of the phenoxy acids do not show significant differences from the recoveries of the other pesticides, which indicates that the reaction is proceeding to completion. Previous work has shown the reaction yields to be ~100% for four of the phenoxy acids.

4. Conclusions

The method described in this paper provides a simple route to the determination of a wide range of pesticides in vegetation samples. Good recoveries have been achieved reproducibly using the extraction procedure with GPC clean-up. Adjustments to the pH of the aqueous phase appears to have little effect on the extraction efficiencies of the majority of the pesticides, the phenoxy acid herbicides were however not extracted at high pH. A multi-residue method involving phenoxy acids must therefore include an adjustment of the pH to ~2, in order to optimize their recoveries. Adjusting the pH of the aqueous phase, first high, then low (see Route A, Fig. 1) does however, appear to produce marginally higher recoveries for a greater number of pesticides compared with the other partitioning procedures studied.

Trimethylsilyldiazomethane has been shown to be an effective reagent for the methylation of phenoxy

Table 3
Percentage recoveries of pesticides from spiked samples of vegetation usingmatrix matched calibration standards

Peak	Pesticides	Recovery from the five modified partitioning methods (A–E) studied (%)				
		A[a]	B	C	D	E
1	Mecoprop	80±1.0	77	65	79	0
2	MCPA	73±4.5	64	56	71	4
3	Dichlorprop	88±1.2	88	76	95	5
4	2,4-D	66±6.2	100	76	103	0
5	Demeton	68±10.5	51	49	42	36
6	Simazine	99±1.3	101	92	95	96
7	Atrazine	101±2.5	107	100	105	102
8	Lindane	77±2.6	56	48	55	62
9	Terbutylazine	96±4.1	109	94	94	98
10	MCPB	81±1.7	116	87	103	0
11	2,4,5-T	14±1.4	21	14	21	0
12	Diazinon	94±2.5	86	79	80	85
13	Disulfoton	67±7.7	53	47	51	53
14	Sebuthylazine	99±2.1	Not analysed			
15	Metobromuron	114±9.2	102	105	101	104
16	Parathion methyl	84±3.2	88	85	86	87
17	Malathion	86±2.8	81	86	84	86
18	Chlorpyriphos	88±3.9	66	79	69	71
19	Cyanazine	112±4.7	103	108	115	112
20	Parathion ethyl	83±3.1	93	89	89	88
21	Metazachlor	103±2.8	108	95	103	102
22	α-Endosulfon	93±4.7	75	72	71	83
23	Ethion	87±1.7	85	89	85	93
24	Azinphos methyl	99±7.0	61	76	67	97
25	cis-Permethrin	86±3.3	82	80	80	95
26	trans-Permethrin	77±4.5	75	73	81	93

[a] Mean of four replicate analyses ($n=4$)±one standard deviation.

acids within a multi-residue method. The reagent does not appear to react with any other pesticides included in this study. The subsequent GC–MS–SIM analysis has enabled sensitive and selective determination of the range of pesticides. Finally the use of matrix matched standards has been shown to be essential for improving the accuracy of results.

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