Derivatisation/solid-phase microextraction followed by gas chromatography–mass spectrometry for the analysis of phenoxy acid herbicides in aqueous samples

Torben Nilsson[a,b,*], Daniela Baglio[a], Isabel Galdo-Miguez[a], Jørgen Øgaard Madsen[b], Sergio Facchetti[a]

[a]*Environment Institute, European Commission Joint Research Centre, I-21020 Ispra (Va), Italy*
[b]*Department of Organic Chemistry, Technical University of Denmark, DK-2800 Lyngby, Denmark*

Abstract

Different combinations of derivatisation and solid-phase microextraction followed by gas chromatography–mass spectrometry were optimised and evaluated for the analysis of phenoxy acid herbicides in water. The most successful derivatisation approach was aqueous-phase derivatisation with benzyl bromide. The benzyl esters were extracted most efficiently by the solid-phase microextraction fibre coated with polydimethylsiloxane–divinylbenzene. No carry-over problems were encountered with this fibre upon desorption at 250°C. Detection limits in the ng/l range were obtained, while the relative standard deviations were between 14 and 42%.   © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Sample handling; Phenoxy acid herbicides; Pesticides

1. Introduction

Previously, successful applications of solid-phase microextraction (SPME) coupled with gas chromatography–mass spectrometry (GC–MS) have been reported for the analysis of a number of organochlorine, organophosphor, and organonitrogen pesticides in aqueous samples [1]. These pesticides were all extracted well by the SPME fibres coated with polydimethylsiloxane (PDMS) or polyacrylate (PA)

after appropriate optimisation, and generally detection limits in the ng/l range were obtainable. The precision and accuracy of the SPME analysis of such pesticides proved very satisfactory in two inter-laboratory studies [2,3]. However, many more polar, thermally unstable and/or less volatile priority pesticides cannot be analysed well by GC without preceding derivatisation [4]. The combination of derivatisation and SPME has been reported for the analysis of phenols [5] and fatty acids [6], but not for the analysis of pesticides. In the present study, different derivatisation/SPME approaches were examined for the GC–MS analysis of the phenoxy acid herbicides 2,4-D, MCPA, dichlorprop and mecoprop. These herbicides are among the ten most important

*Corresponding author. Address for corresponding author: Department of Environmental Science and Engineering, Building 115, Technical University of Denmark, DK-2800 Lyngby, Denmark. Fax: +45-45-932850; E-mail: tn@imt.dtu.dk

pesticides in Europe [7]. The sensitivity and precision were determined after optimisation of the most successful derivatisation/SPME approach.

2. Experimental

2.1. Analytes and sample preparation

The pure analytes 2,4-D, MCPA, dichlorprop and mecoprop were purchased from Dr. Ehrenstorfer (Augsburg, Germany). The purity was above 99% for all of the herbicides. Individual standard solutions and a standard solution containing all of the analytes were prepared by dissolving 100 mg of each herbicide in 40 ml of methanol. The standard solutions were stored at 5°C. A slow methylation of the phenoxy acid herbicides was observed, so the standard solutions should not be stored for periods exceeding one month. For the SPME experiments, samples at concentrations from 10 ng/l to 1 mg/l were prepared in distilled Milli-Q water (Millipore) by spiking with the appropriate amount of the standard solutions shortly prior to analysis. Intermediate dilutions were performed in order to reach the lowest concentrations. The methanol content of the samples for the SPME experiments was kept below 0.1% in all cases in order to avoid interference in the SPME process.

2.2. Derivatisation

Pentafluorobenzyl bromide (PFBBr) (Supelco) and benzyl bromide (Merck) were used for the derivatisation of the phenoxy acid herbicides. All samples were prepared with Milli-Q water in 4 ml screw-cap vials equipped with PTFE coated septa (Supelco) and stirred during the derivatisation and extraction. The samples were heated in an aluminium block (±0.5°C). The following approaches were examined:

2.2.1. Phase-transfer catalysed adsorption to and derivatisation in the polymeric coating of the SPME fibre

The principle of phase-transfer catalysed derivatisation has been described previously for a system containing an aqueous phase and a liquid organic

phase [8,9]. In the present study, the organic phase was the SPME fibre coating. The fibre coating was loaded with benzyl bromide by 10 min equilibration in the vapour of the pure reagent, and subsequently immersed into a 4 ml sample containing 10 mg/l benzoic acid or sodium benzoate as a model compound, 50 mg/l tetrabutylammonium hydrogensulphate and 1 ml of a pH=7.4 phosphate buffer prepared according to Meiring et al. [9]. It was controlled by GC–MS analysis that an excess of benzyl bromide was available for the derivatisation. The reaction was allowed to proceed for up to 5 days at 20°C.

2.2.2. Aqueous-phase derivatisation followed by SPME

The derivatisation conditions were optimised with 3 ml samples containing 1 mg/l phenoxyacetic acid as a model compound. The reaction time was varied from 1 to 16 h, and the reaction was performed at 22, 50 or 80°C. These experiments were performed with 2 μl benzyl bromide and 1 ml of the phosphate buffer. Additional experiments were performed with 10 μl benzyl bromide for comparison, and the influence of pH on the derivatisation was studied with the following conditions: pH adjusted to 3, 4 or 5 with a 0.005 M solution of HCl, natural pH of 6.3, pH adjusted to 7.4 with the buffer or with a 0.005 M solution of NaOH, and pH adjusted to 10.3 with 0.5 g K_2CO_3 or 150 μl of 0.005 M NaOH. The experiments at pH=4, 6.3, 7.4 and 10.3 were carried out both with and without buffer added prior to the extraction. For the purpose of determining the detection limits and carry-over, experiments with the phenoxy acid herbicides at concentrations between 10 ng/l and 1 mg/l were performed with a reaction time of 3 h at 50°C using 3 ml samples containing 1 ml of the phosphate buffer and 2 μl benzyl bromide or PFBBr. After the derivatisation, the samples were cooled to ambient temperature in a water bath and the extraction was initiated immediately.

2.2.3. Combination of headspace-SPME and derivatisation in the polymeric coating

The SPME fibre coating was loaded with benzyl bromide or PFBBr by 10 min equilibration over the pure reagent, and subsequently exposed for 30 min

to the headspace over a 2 ml sample containing 1 mg/l of each phenoxy acid herbicide, 0.35 g/ml NaCl (nearly saturated solution) and a drop of sulphuric acid (pH=0) at 50°C.

2.3. Synthesis of phenoxyacetic acid benzyl ester

The benzyl ester of phenoxyacetic acid was synthesised for the preparation of calibration samples at concentrations corresponding to quantitative derivatisation in order to determine the yield under the different conditions in the experiments with aqueous-phase derivatisation. The synthesis was carried out with 10.0 g phenoxyacetic acid and 14.2 g benzyl alcohol (molar ratio 1:2) in boiling toluene in the presence of 100 mg *p*-toluenesulfonic acid monohydrate until no more water was formed. Subsequently, the toluene was removed using a rotavapor and the remaining acid was neutralised with sodium hydrogencarbonate. Water and diethyl ether were added, and the organic phase was isolated and dried with magnesium sulphate. The diethyl ether was evaporated, and the phenoxyacetic acid benzyl ester was isolated by vacuum distillation at 15 mmHg at approximately 150°C (1 mmHg=133.322 Pa). The purity of the ester was found to be at least 95% by GC–MS analysis.

2.4. SPME procedure for the derivatisation experiments

All SPME fibres (Supelco) were mounted in a SPME holder for manual use (Supelco). The SPME fibre coated with 100 µm PDMS was used in the study of phase-transfer catalysed derivatisation. The headspace-SPME/derivatisation experiments were performed with the SPME fibres coated with 65 µm polydimethylsiloxane/divinylbenzene (PDMS–DVB) and 65 µm carbowax/divinylbenzene (CW–DVB). The GC–MS analyses were performed immediately after the combined SPME/derivatisation experiments. For the purpose of optimising the extraction procedure following the aqueous-phase derivatisation, the SPME fibres coated with 100 µm PDMS, 85 µm PA, 65 µm PDMS–DVB and 65 µm CW–DVB were tested using 3 ml samples, with or

without 0.3 g/ml NaCl, containing 1 mg/l phenoxyacetic acid benzyl ester and 1 ml of the phosphate buffer. The SPME fibre coated with 65 µm PDMS–DVB and no salt addition were used in the further experiments. Extraction times between 5 and 120 min were examined with samples containing 200 µg/l phenoxyacetic acid benzyl ester, and 1 h extraction at 22°C was chosen as standard procedure.

2.5. GC–MS analysis

The desorption was performed for 5 min at 250°C in the injection port of the GC with the split closed, except with the PA coating where the desorption temperature was 290°C. The analyses concerning phase-transfer catalysed derivatisation were performed using a Hewlett–Packard series 5890 GC instrument coupled to a VG Trio 2 quadrupole MS system. A 30 m×0.31 mm I.D. DB-5 column (J&W Scientific) with a film thickness of 1 µm was used with the following temperature program: 120°C for 5 min, then 8°C/min to 280°C which was held for 2 min. The carrier gas was helium with a flow-rate of 2 ml/min. The MS was operated in the electron impact mode, scanning from m/z=20 to 350 in 1 s. In the studies of aqueous-phase derivatisation and headspace-SPME/derivatisation, a Varian GC 3400 coupled with a Finnigan ITS-40 ion-trap MS controlled with the Magnum software (Finnigan) was used. A 30 m×0.25 mm I.D. SPB-5 column (Supelco) with a film thickness of 0.25 µm was used with the following temperature program: 50°C for 5 min, then 20°C/min to 100°C and 10°C/min to 250°C. Helium was used as carrier gas with a column head pressure of 150 kPa. The temperature of the transfer line was 260°C, and the MS was operated in the electron impact mode. In the analyses of phenoxyacetic acid benzyl ester, the scan range was from m/z=50 to 350 in 1 s and the quantitation was based on the molecular ion at m/z=242. Monitoring of multiple single ions was not possible. However, in order to increase the sensitivity the scan range was limited to m/z=285–330 and m/z=375–420, respectively, for the determination of the benzyl and PFB esters of the phenoxy acid herbicides, and the characteristic ions listed in Table 1 were used for the quantitation of the esters.

Table 1
Characteristic ions and SPB-5 retention times for the phenoxy acid herbicide benzyl and PFB esters

Phenoxy acid herbicide	Benzyl ester		PFB ester	
	m/z	Retention time (min)	m/z	Retention time (min)
2,4-D	310	23.0	400	21.3
MCPA	290	22.1	380	20.5
Dichlorprop	324	22.3	414	21.0
Mecoprop	304	21.5	394	20.2

3. Results and discussion

3.1. Optimisation of the derivatisation/SPME procedure

Phenoxy acid herbicides need to be derivatised in order to be analysed well by GC [10]. Furthermore, the extraction efficiency with SPME is expected to be higher for the derivatives. For the combination of derivatisation and SPME, the derivatisation can be performed either in the aqueous phase or in the polymeric fibre coating immersed in the water or exposed to the headspace above it. Thus, inevitably water or moisture will be present during the derivatisation. Silylation and alkylation are the usual derivatisation procedures for compounds containing a carboxyl group [8,11]. Previously, the successful silylation of phenoxy acid herbicides has been reported [12], but since the silylation reagents and derivatives are easily hydrolysed in the presence of moisture [10,13], the combination of silylation and SPME would not be feasible. Previous examples of alkylation of the phenoxy acid herbicides include methylation with diazomethane or boron trifluoride–methanol [13,14] and pentafluorobenzylation [9,14] in organic solvents. However, the use of diazomethane is unattractive because it is extremely toxic, highly irritating and explosive [6,14], and the methylation with boron trifluoride–methanol cannot be combined readily with SPME. For these reasons, and because PFB esters of organic acids are fairly stable in water [6,15], PFBBr was chosen as derivatisation reagent. Besides, the PFB esters will have a higher affinity for the SPME fibre coating than the methyl esters. The pentafluoro analogue was selected in order to enhance the response with an electron-capture detector [8]. However, in MS analyses ordinary benzyl bromide might perform equally well

as or better than PFBBr as derivatisation reagent. Therefore, also benzyl bromide was tested in this study.

The phase-transfer catalysed derivatisation in the polymeric coating of the SPME fibre immersed in the aqueous sample was not successful, possibly because the fibre coating did not extract the ion pairs. However, a small amount of the ester was produced, and it was assumed that the derivatisation took place in the aqueous phase after diffusion of the benzyl bromide from the fibre into the water.

The experiments with aqueous-phase derivatisation confirmed this assumption, although it has been stated previously that carboxylic acids cannot be derivatised directly in the aqueous phase [5]. In the SPME procedure following the aqueous-phase derivatisation, the extraction efficiency was highest with the fibre coated with 65 μm PDMS–DVB (see Fig. 1), and salt addition had a slightly negative
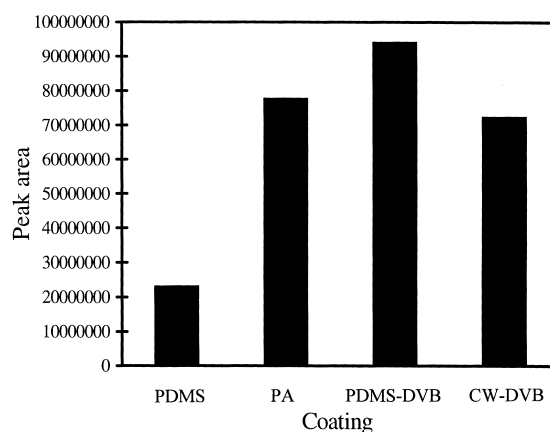

Fig. 1. Response with different SPME fibres after 1 h extraction from a stirred water sample containing 1 mg/l phenoxyacetic acid benzyl ester. No correction for the different volumes of the fibre coatings was performed.

effect. After 1 h extraction under rapid stirring, the response was approximately 70% of the equilibrium value. The carry-over was less than 0.1% in all cases with this fibre. The optimum conditions for the aqueous-phase derivatisation were 3 h reaction at 50°C. Almost identical conditions were used in the aqueous-phase derivatisation of fatty acids by PFBBr [6]. At shorter or longer reaction times and at lower temperature, the yield was lower. At 80°C, the yield was very low after 1 h and no ester was found after 3 h. The maximum is determined as a compromise between two reactions, namely the formation of the ester by benzylation of the acid and degradation of the ester by hydrolysis. Furthermore, the esterification of the acid proceeds in competition with the hydrolysis of benzyl bromide. Increasing the amount of benzyl bromide from 2 to 10 µl had no effect on the yield after 1 and 3 h, while the decrease of the yield at longer reaction times was less rapid. However, more by-products were formed. At pH=10.3, a lower yield was observed than under neutral conditions. This was expected, because at alkaline conditions the hydrolysis of benzyl bromide and the ester is faster. However, the effect was less pronounced than previously observed for fatty acids [6], possibly due to the larger excess of derivatising reagent used in the present study. At pH values down to 3, no major differences in the yields were observed. At strongly acidic conditions, a lower yield of the derivatisation would be expected because the acid is present mainly on the neutral form. However, considering that the pK_a value of phenoxyacetic acid is 3.17 [16], the effect should be of importance only at lower pH values which were not examined due to the risk of damaging the fibre. In the study of fatty acids having pK_a values of approximately 4.8 [16], the effect became clear at pH=4 [6]. The further experiments with aqueous-phase derivatisation were performed at pH=7.4 in order to minimise the hydrolysis. A slightly lower extraction efficiency was observed with the buffer than without.

In the headspace-SPME/derivatisation of the phenoxy acid herbicides, the responses were approximately twice as high with the CW–DVB coating as with the PDMS–DVB coating. However, roughly the response was two orders of magnitude below the that of the aqueous-phase derivatisation. Thus, even at the extreme conditions, i.e. pH=0 and 100% salt

Table 2
Relative standard deviations and detection limits obtained with aqueous-phase derivatisation using benzyl bromide followed by SPME–GC–MS

Phenoxy acid herbicide	R.S.D. (%) [a]	Detection limit (µg/l)
2,4-D	32	1
MCPA	42	0.5
Dichlorprop	18	0.2
Mecoprop	14	0.1

[a] Four replicates at 1 mg/l.

saturation, the phenoxy acid herbicides were still too water soluble and not enough volatile to be extracted well from the headspace.

3.2. Precision and detection limits

The relative standard deviations (R.S.D.s) of four replicates at 1 mg/l and the detection limits observed with aqueous-phase derivatisation using benzyl bromide are given in Table 2. The responses with PFBBr were approximately five times lower. The precision was comparable to previous results with pentafluorobenzylation [14,17]. The R.S.D. of the SPME procedure was only 2% for the benzyl ester of phenoxyacetic acid, while the relatively unstable yield of the derivatisation was the main source of variation. This may also be influenced by matrix effects. The detection limits for the benzyl esters were near 1 ng/l. The reason for the considerably higher detection limits for the phenoxy acid herbicides was the low yield of the derivatisation (~1%).

4. Conclusions

The combination of SPME and derivatisation was complicated by the presence of water. Nonetheless, aqueous-phase derivatisation of the phenoxy acid herbicides with benzyl bromide followed by SPME–GC–MS analysis proved feasible. The SPME–GC–MS analysis of the benzyl esters was very precise and sensitive, whereas the yield of the derivatisation was low and relatively unstable.

References

[1] J. Pawliszyn, Solid-phase Microextraction: Theory and Practice, Wiley–VCH, New York, 1997.

[2] T. Górecki, R. Mindrup, J. Pawliszyn, Analyst 121 (1996) 1381–1386.

[3] R. Ferrari, T. Nilsson, R. Arena, P. Arlati, G. Bartolucci, R. Basla, F. Cioni, G. Del Carlo, P. Dellavedova, E. Fattore, M. Fungi, C. Grote, M. Guidotti, S. Morgillo, L. Müller, M. Volante, J. Chromatogr. A 795 (1998) 371–376.

[4] C. Tomlin (Ed.), The Pesticide Manual, Incorporating The Agrochemicals Handbook, 10th ed., British Crop Protection Council and The Royal Society of Chemistry, Bath, 1995.

[5] K.D. Buchholz, J. Pawliszyn, Anal. Chem. 66 (1994) 160–167.

[6] L. Pan, J. Pawliszyn, Anal. Chem. 69 (1997) 196–205.

[7] M. Fielding, D. Barceló, A. Helweg, S. Galassi, L. Torstensson, P. van Zoonen, R. Wolte, G. Angeletti, Pesticides in Ground and Drinking Water, Water Pollution Research Report 27, Commission of the European Communities, Brussels, 1992.

[8] D.R. Knapp, Handbook of Analytical Derivatisation Reactions, Wiley, New York, 1979.

[9] H.D. Meiring, G. den Engelsman, A.P.J.M. de Jong, J. Chromatogr. 644 (1993) 357–365.

[10] I. Brondz, I. Olsen, J. Chromatogr. 598 (1992) 309–312.

[11] J. Drozd, J. Chromatogr. 113 (1975) 303–356.

[12] M.J. Bertrand, A.W. Ahmed, B. Sarrasin, V.N. Mallet, Anal. Chem. 59 (1987) 1302–1306.

[13] J. Horner, S.S. Que Hee, R.G. Sutherland, Anal. Chem. 46 (1974) 110–112.

[14] J. Hajšlová, W.H. Tahtah, Z. Jehličková, V. Kocourek, P. Cuhra, Sci. Tot. Environ. 132 (1993) 259–274.

[15] F.K. Kawahara, Anal. Chem. 40 (1968) 2073–2075.

[16] J.A. Dean (Ed.), Lange's Handbook of Chemistry, 13th ed., McGraw–Hill, New York, 1985.

[17] V. Lopez-Avila, P. Hirata, S. Kraska, J.H. Taylor Jr., J. Agric. Food Chem. 34 (1986) 530–535.