Original Paper

Comparison of Different Sorbents for Solid-Phase Extraction of Phenoxyalkanoic Acid Herbicides

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Abstract. An HPLC procedure for determination of phenoxyalkanoic acid herbicides in water samples is proposed. The analytical column Phenomenex C18(2) Luna 5 μm and UV detection at 225 nm were applied. Baseline resolution was achieved in isocratic mode with a mobile phase consisting of acetonitrile/acetic acid (40/60, v/v), adjusted to pH 2.5. SPE sorbents – C_{18} BondElut, phenyl-silica, LiChrolut SAX and polymeric sorbents – were compared for isolation and preconcentration of 6 phenoxyalkanoic acid herbicides. Higher (above 95%) and more reproducible recoveries were obtained with polymeric and phenyl-silica sorbents using pure methanol for elution. The method was tested for river water samples with the limit of detection in the range of 2–3 μg L^{-1} (for 50 mL sample) and a reproducibility of 5% RSD.

Key words: Chlorophenoxy acid herbicides; solid-phase extraction; water analysis.

Phenoxyalkanoic acid herbicides are widely used in agriculture to control the growth of broad-leaf weeds in crops. These compounds are considered potential pollutants owing to their high soil mobility, long half-lives and large scale of application. Due to their persistence, polar nature and water solubility, they are present in several environmental compartments including water, soil, cereals and vegetables. Hence, there is increasing interest in methods for the determination of phenoxyalkanoic acid herbicide residues as well as their phenolic metabolites in natural samples.

Herbicides have been extensively analysed by chromatographic [1–10] and electrophoretic methods [10–13]. The standard methods by gas chromatography require the additional step of derivatisation of the carboxyl groups, which is not necessary for HPLC determination. Capillary electrophoresis presents difficulties due to very similar mobility of some herbicides [11]. Nevertheless, the detection limits imposed by environmental quality legislation can usually be achieved by sample preparation techniques which provide high enrichment factors of the target analytes. Solid-phase extraction (SPE) is the most commonly used technique for sample enrichment, because it overcomes many of the disadvantages of liquid–liquid extraction [14, 15]. Several types of sorbents have been developed for the preconcentration of phenoxyalkanoic acid herbicides, those based on chemically bonded silica being the most widely used [16–19]. Polymeric sorbents, in addition to their broader pH stability range, show higher analyte retention for very polar compounds than bonded silica [17, 19, 20]. Chemically modified resins with different functional groups (such as carboxybenzoyl and quaternary

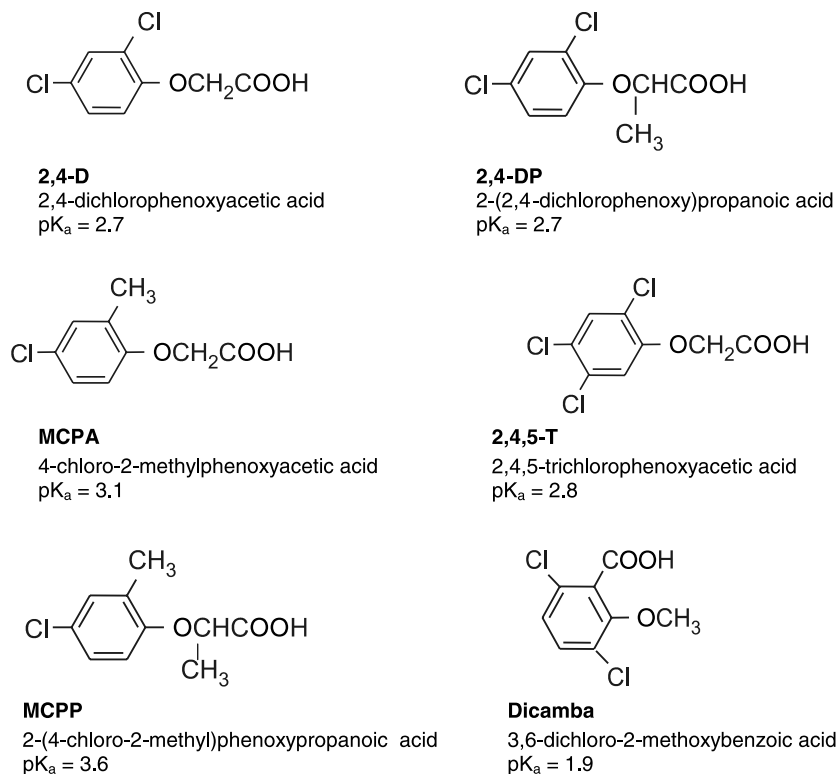* Author for correspondence: E-mail: kryspyrz@chem.uw.edu.pl

2,4-D
2,4-dichlorophenoxyacetic acid
$pK_a = 2.7$

2,4-DP
2-(2,4-dichlorophenoxy)propanoic acid
$pK_a = 2.7$

MCPA
4-chloro-2-methylphenoxyacetic acid
$pK_a = 3.1$

2,4,5-T
2,4,5-trichlorophenoxyacetic acid
$pK_a = 2.8$

MCPP
2-(4-chloro-2-methyl)phenoxypropanoic acid
$pK_a = 3.6$

Dicamba
3,6-dichloro-2-methoxybenzoic acid
$pK_a = 1.9$

Fig. 1. Names, structural formulae and pKa values of investigated chlorophenoxy acid herbicides

ammonium) have also been applied taking advantage of the additional ion exchange mechanism [21, 22].

In this study, different solid sorbents, viz. bonded silica and styrene-divinylbenzene copolymers, have been tested and compared for SPE of 2,4-D, 2,4-DP, 2,4,5-T, MCPA, MCPP and dicamba, the most important phenoxyalkanoic acid herbicides. The extracted analytes were determined by HPLC with UV detection. Names, structural formulae and pKa values of these herbicides are shown in Fig. 1.

Experimental

Apparatus and Reagents

The separations were carried out with an HPLC system from Merck, which consisted of an isocratic pump (type L-7100), an autosampler (L-7250) with a 20 μL loop and a UV detector (L-7400). The data was collected and evaluated using Merck chromatographic software HSM 4.1. The analytical column was a Phenomenex C18(2) Luna 5 μm (250 × 4.6 mm i.d.). The mobile phase consisted of $26.2 \, mmol \, L^{-1}$ acetic acid at pH 2.5 with 40% (v/v) acetonitrile. It was delivered isocratically at $1 \, mL \, min^{-1}$. UV detection was performed at 225 nm. All the assays were carried out at room temperature.

A solid phase extraction vacuum station (Varian Sample Preparation products) was used. SPE was carried out with C_{18} BondElut cartridges (500 mg–3 mL) from Varian, LiChrolut SAX cartridges (500 mg) from Merck, phenyl-silica (500 mg) from Altech, Oasis HLB (30 mg) from Waters and polymeric sorbent Strata-X (30 mg–1 mL) from Phenomenex.

The acid herbicides including 2,4-D, 2,4-DP, MCPA, MCPP, 2,4,5-T and dicamba (see Fig. 1 for names and structural formulae) were obtained as individual standards from Sigma-Aldrich and used for preparing the stock standard solutions.

The high-purity water was taken from a Milli-Q water system (Millipore). Acetonitrile for chromatography from Merck was applied for the preparation of HPLC eluents which were passed through a 0.45 μm membrane filter.

Sample Preparation

SPE cartridges were conditioned by washing successively with 1 mL of methanol and twice with 1 mL of deionised water. Then appropriate volumes of water samples (acidified to pH 2.5) were forced through the cartridge using vacuum. The components were eluted with 1 mL of methanol. The entire extraction procedure was repeated simultaneously at least three times for each kind of tested sorbent material.

This SPE procedure was applied to all cartridge types except the SAX one. In this case, the conditioning step was performed with 1 mL of methanol and then with 1 mL of $26.2 \, mmol \, L^{-1}$ acetic acid solution (to convert the sorbent into the acetate form) and deionised water. The analytes were preconcentrated without acidification, and elution was done using 1 mL of acetonitrile/acetic acid, pH 6.9 (40/60, v/v) mixture.

Results and Discussion

Chromatographic Conditions

For the LC separation of phenoxyalkanoic acids, reversed-phase octadecyl silica is most frequently used. However, the dissociation of these compounds

should be suppressed as far as possible. For this purpose, the mobile phase should be acidified, buffered or contain an ion-pair component. To achieve adequate resolution and analysis time, a gradient elution is often recommended [8, 16, 19, 21]. In our study, isocratic elution and UV detection was performed as it is the simpler method. Phenoxyalkanoic acid herbicides have two local UV absorption maxima at 225–230 nm and at 280 nm. Detection at 225 nm is usually preferred because of higher sensitivity.

A study to select the adequate composition of the mobile phase (pH and organic modifier concentration) was performed using an acetonitrile-acetic acid mixture at pH 2.5 (Fig. 2). As the acetonitrile (ACN) content increased, the k value for the more hydrophobic compounds (2,4,5-T, 2,4-DP and MCPP) decreased. Conversely, the k value for dicamba, the most hydrophilic compound, did not vary. The data indicates that the retention mechanism for dicamba is different than for other compounds studied. The increase in ACN content increases the eluent strength and reduces the hydrophobic interaction between the analyte and the stationary phase. Sanchis-Machols et al. [4] could not obtain adequate resolution of 2,4-D and MCPA or 2,4-DP and 2,4,5-T pairs using the mobile phase at pH 2.5 containing ACN/phosphate buffer (40/60, v/v). Similarly, separation of those herbicide pairs was not achieved using an ion-pair mechanism and gradient elution [16].

When the concentration of acetic acid in the eluent was decreased, an increase in the retention times was observed for all of the investigated compounds. Moreover, the chromatographic peak of MCPA completely
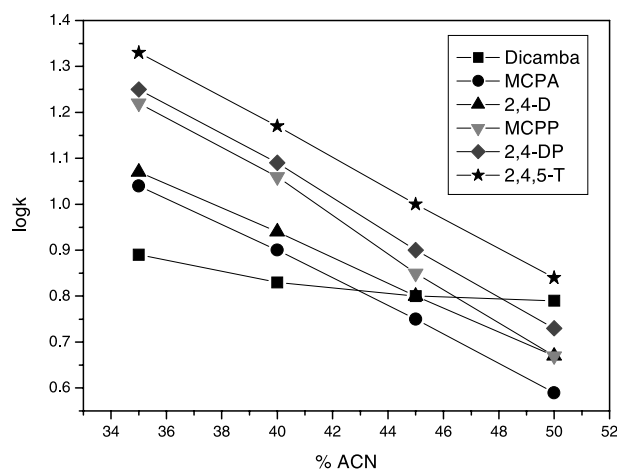
overlapped with 2,4-D, and the resolution factor R_s below one was achieved for dicamba and 2,4-DP herbicides. The pH value of the mobile phase, however, has a more pronounced effect. An increase in the pH dramatically reduces the retention of phenoxyalkanoic acids, particularly in the range of 2.5–3.5, and at pH above 4 only one broad peak was obtained in the chromatogram. These compounds are relatively strong acids (pKa < 4), almost completely dissociated in water, and are not retained by reversed-phase C_{18} in the ionised state. In order to increase the retention capacity, the pH of the eluent should be lower than the pKa of the analytes. However, the pH of an acidic mobile phase used in RP-LC of acid herbicides is a compromise between the separation efficiency and the decrease in the column lifetime. Thus, a mobile phase composition with 40% acetonitrile/60% CH_3COOH aqueous solution (26.2 mmol L^{-1}) at pH 2.5 was selected. Using this eluent, the retention times of the more hydrophobic herbicides were low enough, and total baseline resolution was achieved.

The calibration curves of compounds were obtained by triplicate injections of standard solutions containing different concentrations of the analytes. The peak area was plotted versus analyte concentration. All compounds showed good linearity in the 50–1000 μg L^{-1} range. A preliminary preconcentration step is necessary for the determination of lower concentrations of phenoxyalkanoic acid herbicides.

Comparison of Tested SPE Materials

To meet the objectives for the monitoring of acidic chlorophenoxy herbicides in water samples, the performance of C_{18} BondElut, phenyl-silica, LiChrolut SAX and polymeric Strata-X as well as Oasis were compared. Several types of sorbents have been reported [16–22] for isolation and enrichment of phenoxyalkanoic acids, yet the efficiencies of the proposed methods have rarely been compared.

Octadecyl silica is the sorbent most frequently reported for its use in the isolation of all types of acidic herbicides. The mechanism of retention with such sorbents depends on Van der Waals forces, hydrogen bonding or dipole–dipole interactions. Polymeric sorbents such as styrene-divinylbenzene copolymers have a broader pH stability range and a greater surface area. Thus, their application increases the flexibility of the SPE method. An additional advantage of polymeric sorbents is selective π–π interaction with analytes containing aromatic rings. The Oasis HLB
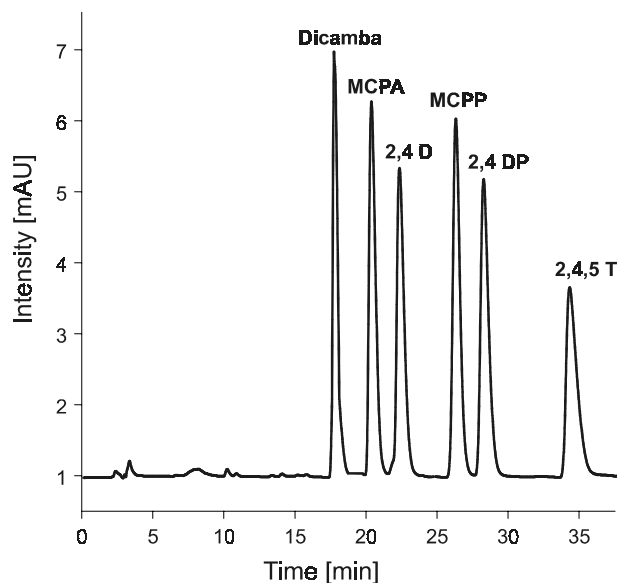

Fig. 2. Effect of acetonitrile content in the mobile phase on the retention of chlorophenoxy acid herbicides

Fig. 3. Chromatogram of the chlorophenoxy acids. Column: Phenomenex C18(2) Luna 5 μm (250 × 4.6 i.d.); eluent: acetonitrile/acetic acid, 26.2 mpL L^{-1} (60/40, v/v); flow rate 1 mL min^{-1}; UV detection at 225 nm

phase is formed by the macroporous copolymer poly(vinylbenzene-co-N-vinylpyrrolidone) which exhibits both hydrophilic and liphophilic retention characteristics [19]. The two main features of this reversed-phase sorbent are the ability to be used with pure water and to retain a wide spectrum of both polar and non-polar compounds. Phenyl-silica exhibits a slightly different selectivity from other non-polar sorbents, which derives from the electron density of the aromatic ring. With the use of a strong anion exchanger, different mechanisms can be involved in the retention process of the analytes. In acidic media, the predominant retention mechanisms are $\pi-\pi$ and Van der Waals interactions. Conversely, the ion-exchange mechanism predominates at high pH values when the acid-base equilibrium is clearly shifted to the ionic form.

The extraction efficiency of the SPE sorbents was evaluated by spiking 10 mL of deionised water with 50 ng of each phenoxy herbicide. The enrichment of these herbicides on hydrophobic sorbents requires acidic conditions, thus, the pH of the samples was adjusted to 2.5 (with glacial acetic acid). To achieve optimum extraction conditions using the strong anion exchanger, i.e. LiChrolut SAX, this sorbent and the analytes should be oppositely charged. Thus, for the enrichment step the acidic compounds were kept in the proper ionised form (at neutral pH) for retention
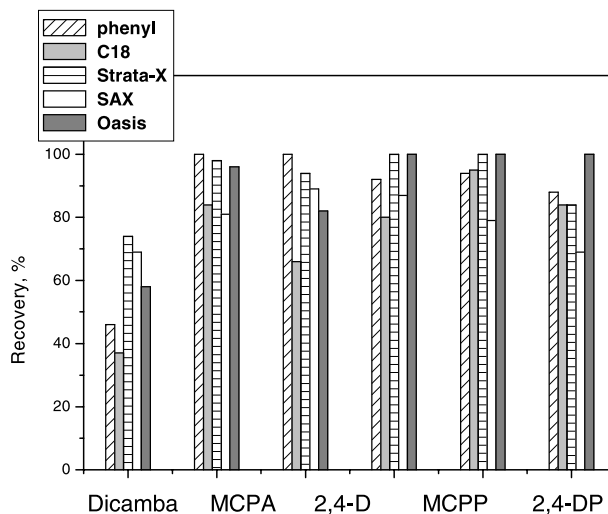

Fig. 4. Recoveries of chlorophenoxy acids extracted from 10 mL of deionised water spiked at the 5 μ L^{-1} level using various SPE cartridges

on the anion-exchange cartridge. The obtained results are presented in Fig. 4.

The recovery of the investigated compounds was higher for polymeric Strata-X, Oasis and phenyl-silica sorbents. The better performance of these sorbents in comparison with silica gel C_{18} can be attributed to their aromatic structure, which can interact with aromatic analytes *via* $\pi-\pi$ interactions. Additionally, Oasis HLB cartridges are water-wettable, and thus there is no need to ensure that it remains wet before loading the aqueous sample. The recovery of dicamba, the least hydrophobic compound evaluated, was much lower; only its sorption on Strata-X reached 74%. Peruzzi et al. [19] reported that 2,4-D, 2,4,5-T and dicamba were not recovered from the polymeric LiChrolut ENU sorbent, but they preconcentrated the water samples without pH adjustment. Puig and Barceló [23] also found that polymeric sorbents are the most suitable materials for the recovery of the whole range of polar phenols at pH 2.5.

C_{18} silica sorbent was found to be less appropriate for isolation and preconcentration of the tested herbicides. Nevertheless, some of the analytes displayed recoveries higher than 90%. The sorbent efficiency decreases as compound polarity increases. The application of acetonitrile/acetic acid solution (40/60, v/v) at pH 6.9 did not produce better results.

The retention of phenoxyalkanoic acid herbicides on a strong anion exchanger under the experimental conditions was complete, but elution using acetonitrile/acetic acid solution at neutral pH exhibits

low efficiency, thus, the whole recovery process gave unsatisfactory results (Fig. 4). The elution of analytes from this type of sorbent should be favoured with the increase of the eluent acidity. This approach was found to be useful for increasing about three times the recovery of the tested compounds; however, the obtained results were not as good as for the polymeric sorbent, and their reproducibility was very poor. One of the main problems in SPE procedures is the compatibility between the eluents used for analyte desorption and chromatographic analysis. In our approach it is not advisable to use strong acid with organic solvent mixtures for elution of retained compounds and introduce them into a silica gel C_{18} analytical column.

Recoveries of phenoxyalkanoic acid herbicides extracted from larger sample volumes at the $5 \mu g L^{-1}$ level are shown in Table 1. Dicamba is not effectively trapped resulting in break-through when more than 50 mL of sample is preconcentrated. Polymeric

Strata-X and Oasis HLB are the sorbents with the strongest retention among the materials investigated.

To evaluate the proposed method, water samples were collected from Oligocene well source and from Vistula river near Warsaw, Poland. The samples were filtered through a $0.45 \mu m$ membrane filter to eliminate the suspended solid particles and spiked with herbicide stock solution giving a concentration of $5 \mu g L^{-1}$ for each compound. 100 mL of these samples were then passed through preconditioned Oasis HLB, phenyl-silica and Strata-X SPE cartridges. Figure 5 shows the obtained chromatograms. The recovery values of determined compounds were in the range of 78–102% for groundwater and 79–104% for river water. The highest recovery for all studied phenoxyalkanoic herbicides was obtained for Oasis HLB sorbent. The reproducibility, expressed as the relative standard deviation, was 5% at the most (n = 4). The limit of detection (LOD) for the proposed method was determined by analyzing the spiked river water

Table 1. Recoveries [%] of phenoxyalkanoic herbicides preconcentrated from different sample volumes

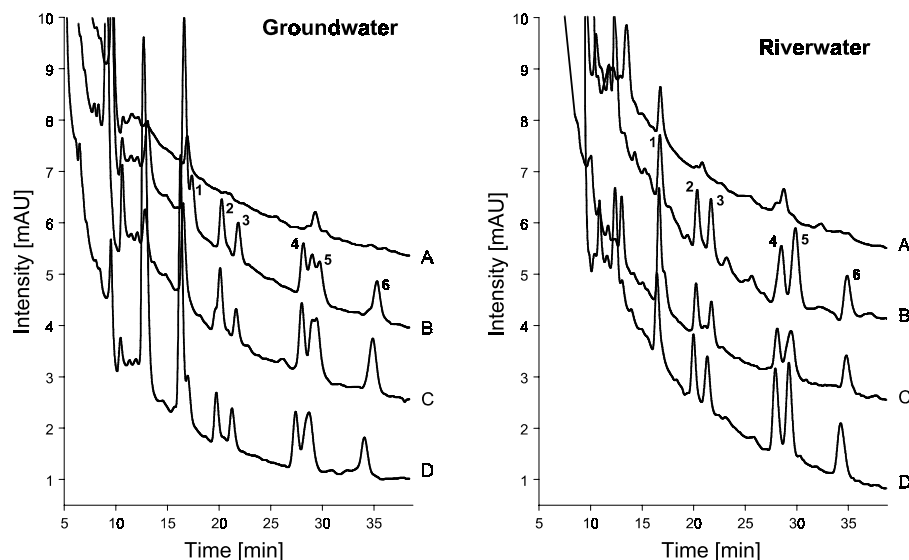| Sorbent	Dicamba		MCPA		2,4-D		MCPP		2,4-DP		2,4,5-T	
	Sample volume, mL											
	50	100	50	100	50	100	50	100	50	100	50	100
Phenyl	13	10	100	95	88	82	82	69	75	74	88	84
C18	21	8	78	53	57	45	70	47	94	77	61	52
Strata	62	55	99	92	92	88	94	86	99	93	79	77
SAX	50	35	57	36	70	60	68	53	69	39	58	55
Oasis	47	42	95	88	82	80	92	91	98	98	94	92

Mean value (n = 3).

Fig. 5. Chromatograms obtained by preconcentration of groundwater (from oligocene aquifer) and river water samples, spiked at the $5 \mu g L^{-1}$ level with phenoxyalkanoic acids. Peak identification: (*1*) dicamba, (*2*) MCPA, (*3*) 2,4-D, (*4*) MCPP, (*5*) 2,4-DP, (*6*) 2,4,5-T. (*A*) Preconcentrated without spiking; (*B*) after spiking and preconcentrated using Oasis; (*C*) Strata-X; (*D*) phenyl-silica cartridges

samples at decreasing concentrations of target compounds until the response obtained had a peak height three times larger than the average height of noise around the peak. The LOD values were in the range of 2–3 $\mu g\,L^{-1}$ for 50 mL samples and are better or similar to those reported by Menor-Higueruelo et al. [22] when a BondElut C_{18} solid phase extraction column was used.

One of the major problems encountered in the pre-concentration of acidic compounds from natural water samples is that some of the matrix components could also be co-extracted, resulting in a broad peak at the beginning of the chromatogram [17, 19, 23]. This peak can interfere with the quantification of the analytes of interest. In our approach, the chromatographic peak corresponding to the polar substances does not overlap with the dicamba peak. Thus, clean-up strategies are not necessary.

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