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Molecularly imprinted solid-phase extraction sorbent for the clean-up of chlorinated phenoxyacids from aqueous samples

Claudio Baggiani*, Cristina Giovannoli, Laura Anfossi, Cinzia Tozzi

Dipartimento di Chimica Analitica, Università di Torino, Via Pietro Giuria 5, I-10125 Turin, Italy

Abstract

A molecularly imprinted polymer (MIP) was synthesized using the herbicide 2,4,5-trichlorophenoxyacetic acid as a template, 4-vinylpyridine as an interacting monomer, ethylendimethacrylate as a cross-linker and a methanol–water mixture as a porogen. The binding properties and the selectivity of the polymer towards the template were investigated by frontal and zonal liquid chromatography. The polymer was used as a solid-phase extraction material for the clean-up of the template molecule and some related herbicides (2,4-dichlorophenoxyacetic acid, fenoprop, dichlorprop) from river water samples at a concentration level of ng/ml with quantitative recoveries comparable with those obtained with a traditional C_{18} reversed-phase column when analyzed by capillary electrophoresis. The results obtained show that the MIP-based approach to the solid-phase extraction is comparable with the more traditional solid-phase extraction with C_{18} reversed-phase columns in terms of recovery, but it is superior in terms of sample clean-up. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

The widespread use of chlorinated phenoxyacids as selective herbicides has led to increased food, soil and water pollution by their residues. Due to the high toxicity for mammals and aquatic organisms and the long-term persistence in the environment, legislation poses severe limits on the presence of these pesticides as residues and make them a suitable subject of trace analysis.

The detection and quantification of chlorinated phenoxyacids at trace levels cannot be performed directly using gas chromatography, high performance liquid chromatography or capillary zone electrophoresis, but it requires one or more preconcentration steps, due to the insufficient sensitivity of these analytical methods [1–5].

The solid-phase extraction could represent a suitable way to clean-up and preconcentrate samples containing traces of chlorinated phenoxyacids. This technique is more rapid, simple, economical and environment-friendly than the traditional liquid–liquid extraction. The main problem associated with solid-phase extraction columns packed with ordinary stationary phases (such as ion-exchange or reversed-phase materials) is the low selectivity of the retention mechanism. Also with the desired analyte(s), many interfering substances of similar hydrophobicity/hydrophilicity are retained and concentrated, due to the very limited selectivity of the partition equilibria involved [6,7].

A desired grade of selectivity may be obtained

*Corresponding author. Tel.: +39-011-670-7622; fax: +39-011-670-7615.

E-mail address: baggiani@ch.unito.it (C. Baggiani).

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using columns packed with materials based on well-defined molecular recognition mechanisms, which are able to bind the desired analyte with a high grade of selectivity, such as immunoaffinity columns. Even though well described in the literature [8–11], these kinds of solid-phase extraction (SPE) columns are affected by problems that limit a more general use in the environmental field of analysis. High production costs for specific antibodies from polyclonal or monoclonal antisera, and the poor stability of the antibody binding sites in the organic solvents, necessary to quantitatively recover the analyte from the solid-phase after the washing step, make a large-scale use of these sorbents impracticable.

Solid-phase extraction procedures involving molecular imprinted polymers (MISPE) have been proposed as a valid substitute of immunoaffinity procedures, due to their simplicity of preparation and high stability to extreme chemical conditions [12]. Efficient extraction procedures have been described in the literature for pesticides in water and other complex matrices [13–18].

In this work we use a polymer obtained by imprinting with the herbicide 2,4,5-trichlorophenoxyacetic acid, whose molecular recognition properties were described previously [19], as a molecular imprinted solid-phase extraction sorbent. This polymer is used to extract and concentrate the imprinted molecule and other closely related phenoxy acids from river water samples before the analysis by capillary electrophoresis, and a comparison was made with liquid–liquid extraction and reversed-phase solid-phase extraction.

2. Experimental

2.1. Materials

2,4,5-Trichlorophenoxyacetic acid (2,4,5-T), 2,4-dichlorophenoxyacetic acid (2,4-D), 4-chlorophenoxyacetic acid (4-CPA), 2-methyl-4-chlorophenoxyacetic acid (MCPA), (±)-2-(2,4,5-trichlorophenoxy)propionic acid (fenoprop), (±)-2-(2,4-dichlorophenoxy)propionic acid (dichlorprop), (±)-2-(2-methyl-4-chlorophenoxy)propionic acid (mecoprop), atrazine (2-isopropylamino-4-ethylamino-6-chloro-s-triazine), bentazone (3-isopropyl-1-tia-2-benzo-3-diazin-4-one-2,2-dioxide), ethylene dimethacrylate and 4-vinylpiridine were from Sigma–Aldrich–Fluka (Milan, Italy), all other reagents and organic solvents were supplied by Merck (Darmstadt, Germany).

4-Vinylpyridine and ethylene dimethacrylate were distilled at reduced pressure immediately before use. Phenoxyacid stock solutions were prepared by dissolving 10 mg of substance in 20 ml of acetonitrile and stored in the dark at −20°C. Working solutions were prepared in different solvents directly diluting the stock solutions immediately before use. The water of the River Po was taken in December 2000 in Turin in a single sampling, filtered on 0.22-μm polyethylene filters, and stored in polyethylene bottles in the dark at 4°C. The HPLC apparatus (pump L-6200, UV–Vis detector L-4200 and integrator D-2500) came from Hitachi–Merck (Darmstadt, Germany). The capillary electrophoresis apparatus was a Biofocus 2000 (Bio-Rad Labs., Hercules, USA) provided with a UV–Vis detector and interfaced with a Pentium personal computer.

2.2. Reversed-phase liquid chromatography

A monolithic octadecyl-silica column (Chromolith Performance RP-18, 100×4.6 mm, Merck) was used as a stationary phase in reversed-phase liquid chromatography of the samples containing atrazine. The mobile phase consisted of methanol–water (1:1, v/v), acidified with 0.1% (v/v) of acetic acid. The mobile phase flow-rate was set to 1.00 ml/min. Chromatograms were recorded at 280 nm. A calibration curve for the herbicide atrazine was drawn between 1 and 50 μg/ml.

2.3. Capillary zone electrophoresis

Separations were carried out using a phosphate–borate buffer (50 m*M* sodium phosphate monobasic, pH corrected to 7.5 with sodium tetraborate, 2.75 m*M* α-, β-, and γ-cyclodextrin). The buffer was passed through a 0.22-μm polyethylene filter and degassed under sonication. Before each run, the uncoated fused-silica capillary (Machery–Nagel, Düren, Germany), 50 cm (45.4 cm from the detector)×50 μm I.D., was rinsed with sodium hydroxide 0.1 *M* for 90 s, with water for 120 s and then with the run buffer again for 120 s. The samples

were hydrodynamically (0.07 MPa/s) injected into the capillary thermostatically set at 25°C. The applied voltage was 20 kV with a current of about 60 μA. UV detection at 200 nm was performed at the cathodic end of the capillary. Calibration curves for the herbicides were drawn between 0.25 and 25 μg/ml. The corrected peak areas were calculated using BioFocus Integrator 5.0 software.

2.4. Polymer preparation

In a 10-ml-thick wall glass test tube a solution was prepared by dissolving 0.600 g (2.35 mmol) of 2,4,5-T into 3.20 ml of methanol–water (3:1, v/v). Then, 0.243 ml (2.35 mmol) of 4-vinylpiridine, 2.95 ml (15.7 mmol) of ethylene dimethacrylate and 0.040 g of 2,2′-azobis-(2-methylpropionitrile) were added under sonication. The mixture was purged with nitrogen, the tube was sealed, then the mixtures were left to polymerize overnight at 60°C in a waterbath. The monolith obtained was broken, mechanically ground in a mortar and wet-sieved to 30–90 μm particle size. The particulate was extensively washed with methanol–acetic acid (9:1, v/v) till no template was found in the washing solution. No efforts were made to measure the amount of template molecule recovered. A blank polymer was prepared and treated in the same manner, omitting the template.

2.5. Chromatographic evaluation of the imprinted polymer

Adequate amounts of imprinted and blank polymers were suspended in a methanol–water (1:1, v/v) mixture and the slurry packed at a constant pressure of 10 MPa in 100-mm stainless-steel HPLC columns (I.D. 3.9 mm, geometrical volume 1.19 ml). The packed columns were washed at 1 ml/min with methanol–acetic acid (4:1, v/v) until a stable baseline was reached (283 nm).

To evaluate the selectivity of the imprinted polymer, the columns were equilibrated at a flow-rate of 1 ml/min with 40 ml of methanol–acetic acid (99:1, v/v); then, 20 μl of a 5 μg/ml solution of herbicide in methanol were injected and eluted at 1 ml/min, recording the absorbance at 286 nm. Each elution was repeated three times to assure the chromatogram reproducibility. Column void volume was measured by eluting 20 μl of 0.1% (v/v) acetone in methanol, and the absorbance recorded at 286 nm. The retention factor (k') was calculated as $(t-t_o)/t_o$, where t is the retention time of the eluted substance, and t_o the retention time corresponding to the column void volume. The selectivity factor (α) was defined as an index of polymer selectivity towards analogues of the template molecule. It was calculated as $k'_{analogue}/k'_{template}$.

The capacity of the imprinted polymer to retain 2,4,5-T was studied by frontal liquid chromatography, eluting the imprinted column with methanol–acetic acid (99:1, v/v) which contained concentrations of herbicide between 8 and 3000 μg/l. For each elution experiment the column was equilibrated with 30 ml of the eluent; then the pumps and the eluent tubes of the chromatographic apparatus were filled with eluent containing 2,4,5-T, purging out the excess eluent. The elutions were performed at 1 ml/min, recording the absorbance at 230 (herbicide concentration lower than 100 μg/ml) or 283 nm (herbicide concentration greater than 100 μg/ml). After each run the column was washed extensively with methanol–acetic acid (4:1, v/v). The breakthrough volume was measured by the elaboration of the digital signal which is the maximum numerical value of the first derivative of the curve corresponding to the frontal chromatogram.

2.6. Characterization of the MISPE column

An adequate amount of dry imprinted polymer (0.50 g) was suspended in methanol, sonicated in a water-bath for 10 min and packed in a 5-ml empty polypropylene SPE cartridge provided with frits to secure the packing, and an outlet stopcock. The column was connected to a vacuum manifold and washed extensively with glacial acetic acid, methanol–acetic acid (1:1, v/v), methanol, and then dried under vacuum. As a check, a SPE column was prepared in the same manner but with the blank polymer.

In the next experiments, each extraction was repeated three times and analyte recovery was evaluated as the average of the single values measured.

To evaluate the bleeding of residual template molecules from the MISPE column, the dry column

was washed with 100 ml of methanol–acetic acid (4:1, v/v). Then the collected eluate was completely evaporated under vacuum and redissolved in 200 μl of acetonitrile–water (4:1, v/v) under sonication prior to analysis by capillary zone electrophoresis.

To evaluate the effect of the mobile phase on MISPE extraction, dry columns were washed with 10 ml of methanol–acetic acid (4:1, v/v), conditioned with 5 ml of a suitable solvent, followed by 1 ml of 10 μg/ml of the working solution prepared in the same solvent. Then the collected eluate was completely evaporated under vacuum and redissolved in 200 μl of acetonitrile–water (4:1, v/v) under sonication prior to analysis by capillary zone electrophoresis.

To evaluate the effect of different volumes of solvent during the washing of the MISPE column and the elution of the herbicides, 10 ml of deionized water containing 2 μg/ml of 2,4,5-T, 2,4-D, fenoprop, dichlorprop, MCPA, mecoprop, atrazine or bentazone were loaded in a column previously equilibrated with 1×5 ml of deionized water. After the sample loading, air was passed through the column for 10 min, then it was washed with 1×3 ml of methanol and the herbicide recovered with 1×3 ml of methanol–acetic acid (4:1, v/v). Then the collected fractions were unified and treated as previously described. Fractions obtained by eluting atrazine were analyzed by reversed-phase liquid chromatography.

2.7. MISPE of real samples

River water (1 l) was acidified to pH 4 with 1 *M* hydrochloric acid and spiked with 4, 10 and 40 μl of 2,4,5-T, 2,4-D, fenoprop and dichlorprop stock solutions; 100 ml of each sample containing 2–20 ng/ml of each herbicide were loaded into the MISPE column, previously equilibrated with 1×5 ml of deionized water. After the sample loading, air was passed through the column for 10 min. Then it was washed with 1×3 ml of methanol and the herbicide recovered with 1×2 ml of methanol–acetic acid (4:1, v/v). Subsequently the collected fractions were unified and treated as previously described. The same procedure was performed on the blank column.

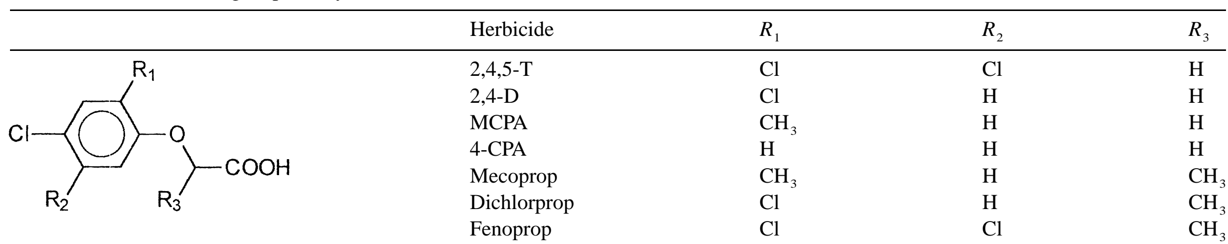
To perform a comparison with the MISPE method, C_{18} solid-phase and liquid–liquid extractions were performed on the same amount of water spiked with the herbicides. In the first experiment, the reversed-phase column was previously equilibrated with 1×5 ml of deionized water. After the sample loading, air was passed through the column for 10 min. Then it was washed with 1×3 ml of water–methanol (9:1, v/v) and the herbicide recovered with 1×2 ml of methanol–acetic acid (99:1, v/v). In the second experiment, the sample was extracted three times with 100 ml of dichloromethane, the organic phases unified and completely evaporated under vacuum. For each method the collected eluates were treated as previously described. The extraction procedures were repeated five times on each sample to evaluate the reproducibility of the methods considered.

3. Results and discussion

3.1. Polymer selectivity

The selectivity of the imprinted polymer was evaluated by liquid zonal chromatography, by eluting several 2,4,5-T-related substances of environmental significance, whose molecular structures are reported in Table 1. Considering the retention and the selec-

Table 1
General structure of halogenophenoxy acids herbicides considered in this work



	Herbicide	*R*$_1$	*R*$_2$	*R*$_3$
	2,4,5-T	Cl	Cl	H
	2,4-D	Cl	H	H
	MCPA	CH_3	H	H
	4-CPA	H	H	H
	Mecoprop	CH_3	H	CH_3
	Dichlorprop	Cl	H	CH_3
	Fenoprop	Cl	Cl	CH_3

Table 2
Retention factors (k') and selectivity factors (α) obtained for the imprinted and blank polymer from liquid chromatography

Herbicide	k' (imprinted)	k' (blank)	α
2,4,5-T	4.39	0.44	1
2,4-D	3.00	0.27	0.68
Fenoprop	2.72	0.39	0.62
Dichlorprop	2.22	0.23	0.51
MCPA	1.38	0.55	0.31
Mecoprop	1.22	0.27	0.28
4-CPA	0.72	0.17	0.16

tivity factors reported in Table 2, it is possible to observe that molecules different in their substitution on the aromatic ring or in the phenoxy sub-structure are recognised proportionally through the similarity with 2,4,5-T. Thus, 2,4-D (chlorine in positions 2 and 4, no chlorine in position 5) are recognised better than 4-CPA (chlorine in position 4, no chlorine in positions 2 and 5). The substitution of a chlorine atom with a methyl group also reduces recognition. Thus, 2,4-D is better recognised than MCPA (chlorine in position 4, methyl in position 2). The same happens with 2-phenoxypropanoic acids: fenoprop (chlorine in positions 2, 4 and 5) is less recognised than 2,4,5-T, but is better recognised than dichlorprop (chlorine in positions 2 and 4, no chlorine in position 5), and dichlorprop is better recognised than mecoprop (chlorine in position 4, methyl in position 2, no chlorine in 5). These results, obtained in methanol–acetic acid used as a mobile phase are very closely related to those obtained utilizing acetonitrile as a mobile phase, as reported in a previous paper [19].

3.2. Polymer binding capacity

To verify the capacity of the imprinted polymer to retain the template molecule, measurements of breakthrough were made in frontal liquid chromatography. In this kind of chromatography, the affinity constant of the polymer for the template, the amount of binding sites in the polymer and the presence/absence of several distinct classes of binding sites contribute to determine the position of the breakthrough point on the time axis in the chromatogram [20]. Thus, from an adequate number of experimental breakthrough points, measured in a well-defined range of template concentrations, it is possible to measure the binding capacity of the imprinted polymer, i.e. the affinity and the binding sites concentration, and also to obtain information on the nature of the binding sites, i.e. the presence of multiple binding site classes.

The experimental breakthrough points obtained, some of which are reported in Fig. 1, were plotted against the corresponding amount of template retained and fitted with several absorption isotherms. While single-site and double-site Langmuir models failed to fit the experimental data and showed a lack of fit for low concentration of herbicide, the best fit was obtained with a Freudlich isotherm, reported in Fig. 2. While the physical meaning of the binding isotherm lies outside the aim of this work, this model is sufficient to show that the imprinted polymer has a good capacity for the template, and that it is suitable for the purpose of solid-phase extraction involving the molecular imprinting mechanism. In particular, from Fig. 1, considering concentrations in the mobile phase of less than 10 μg/ml, a column with a stationary phase volume of 0.50 ml is able to retain almost 30 μg of herbicide without any significant leaching.

3.3. Bleeding of the MISPE column

The potential slow release of residual template molecules from the imprinted column could affect

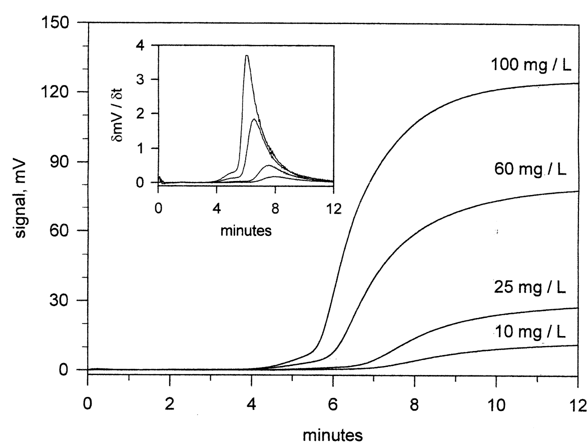


Fig. 1. Imprinted column breakthrough of 2,4,5-T at different concentrations in methanol–acetic acid (99:1, v/v). In the inset: first derivative of the breakthrough curves.

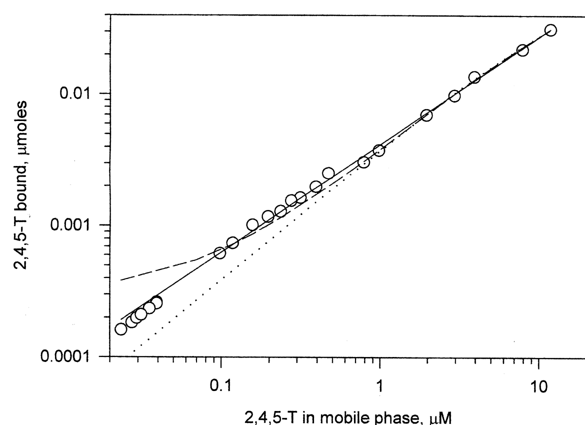


Fig. 2. Experimental isotherm data obtained with the imprinted column and curves obtained fitting the data with a single site Langmuir model (dotted line), with a double site Langmuir model (dashed line) and a Freundlich model (solid line). The axes are reported in logarithmic scale to show the lack of fit for single and double site Langmuir models at low concentration of 2,4,5-T in mobile phase.

the performances of the extraction procedure, thus, residual 2,4,5-T was searched washing the column with methanol–acetic acid to promote the release. At the level of sensitivity obtained by the capillary electrophoresis method used (0.25 µg/ml), considering a concentration factor of 500 obtained by evaporation of the sample and redissolution in 200 µl of running buffered we did not observed any detectable amount of template. Presuming a quantitative recovering in the concentration step, it could be stated that if residual release is present, its magnitude is under the 0.5 ng/ml level, lower than the concentrations of herbicides studied in the extraction of real samples (vide infra).

3.4. Effect of the mobile phase on MISPE extraction

The binding of the template 2,4,5-T and of the closely related herbicide 2,4-D was tested using several organic solvents in common use in solid-phase extraction techniques in order to select suitable solvents for loading, washing and elution. The amount of herbicides not retained by the columns is reported in Tables 3 and 4. As reported in a previous paper [19], the binding mechanism of the imprinted polymer is based on a reversed-phase interaction between the binding sites present in the polymeric matrix and the herbicide molecules. This interaction is modulated by the presence of an ion pair between the pyridinic ring in the binding site and the carboxyl of the interacting molecule. It follows that the polarity of the solvent directly influences the partition of the herbicides in the MISPE column, so that the column behaves like a reversed-phase system in which an increasing hydrophobicity of the mobile phase causes a decrease in the binding capacity. The marked difference of binding between the blank polymer that totally loses its binding properties when the hydrophilic ethanol was used as solvent, and the imprinted polymer shows that the presence of highly hydrophobic binding sites generated by the imprinting process and complementary to the most hydrophobic part of the imprinted molecule, is fundamental to confer binding properties to the polymer.

The decrease of the binding capacity in the

Table 3
Binding of 2,4,5-T and 2,4-D (20 µg in 1 ml) to imprinted and blank polymers in different solvents

Solvent	Imprinted column, % not retained		Blank column, % not retained	
	2,4,5-T	2,4-D	2,4,5-T	2,4-D
Water	n.d.	n.d.	n.d.	n.d.
Methanol	n.d.	n.d.	20±1.5	41±2
Ethanol	n.d.	n.d.	103.5±13.5	96.5±4
1-Propanol	n.d.	n.d.	96.5±6.5	106±8.5
Acetonitrile	n.d.	23.5±7.5	43.5±3.9	96±5.5
Tetrahydrofuran	52.5±2.5	67±4.5	98.5±10.5	100.5±3
Chloroform	74.5±12	103.5±13	91.5±21	99.5±4
Dichloromethane	76±5.5	102±4.5	98.5±6.5	101±1.5

n.d., not detectable.

Table 4
Effect of acetic acid on the binding of 2,4,5-T and 2,4-D (20 μg in 1 ml) to imprinted and blank polymers

% (v/v) of acetic acid in methanol	Imprinted column, % not retained		Blank column, % not retained	
	2,4,5-T	2,4-D	2,4,5-T	2,4-D
0	n.d.	n.d.	20±1.5	41±2
0.1	n.d.	n.d.	34.5±3.5	42.5±1.5
0.5	n.d.	n.d.	42±1	52.5±1.5
1	13±1	26.5±3	54±3	83.5±2.5
2	34.5±4.5	46±2.5	73.5±3	93±3.5
5	51.5±7	61±2	86±4	100.5±1
10	82.5±1	101±2	98±2	98.5±2
20	100.5±2	99.5±2.5	101±1.5	101±2

n.d., not detectable.

presence of acetic acid when methanol was used as a solvent is again a consequence of the mechanism of molecular recognition, in which molecule, able to interfere with the ion pair formation between the pyridine ring and the carboxyl, should reduce the retention of the herbicides on the MISPE column. This fact implies that the overall molecular recognition of the herbicide molecule by the polymer can be efficient only if the electrostatic ion pair interaction between the pyridine ring of the polymer and the carboxyl of the herbicide molecule are enhanced and stabilised by the hydrophobic interaction between the imprinted polymer and the aromatic part of the phenoxyacid. In the blank polymer, the absence of specific binding sites, provided both with hydrophobic character and exposed pyridyl groups, causes a lack of binding capacity, and promotes rapid elution of the analytes when limited amounts of acetic acid are present in the solvent.

Considering the aqueous character of the matrix from which chlorinated phenoxyacids should be extracted, the results obtained show that it is possible to directly load the sample on to the MISPE column without loss of analytes, wash off the potential interferents with a polar solvent such as methanol or ethanol and recover the herbicides through elution with the same solvent containing a relevant amount of acetic acid.

3.5. Effect of washing and elution volumes on MISPE extraction

From the recovery values reported in Table 5, it is clear that the MISPE is suitable for the selective and quantitative extraction of several halogenophenoxy acids from aqueous samples. In fact, the template and the closely related molecules 2,4-D, fenoprop and dichlorprop nearly show quantitative recoveries

Table 5
Herbicide recoveries on MISPE column using 10 ml of deionized water containing 2 μg/ml of analytes

	Herbicides, % of recovery for 20 μg in 10 ml of deionized water							
	2,4,5-T	2,4-D	Fenoprop	Dichlorprop	MCPA	Mecoprop	Atrazine	Bentazone
Washing, 1st ml	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	98.5±3	98±2
Washing, 2nd ml	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Washing, 3rd ml	n.d.	n.d.	n.d.	n.d.	6.5±2.5	10.5±3.5	n.d.	n.d.
Elution, 1st ml	97.5±4	98.5±3.5	96.5±4	95±7.5	87±7	88.5±8	n.d.	n.d.
Elution, 2nd ml	6±2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Elution, 3rd ml	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
% of recovery	103.5±6	98.5±3.5	96.5±4	95.0±7.5	87.0±7	88.5±8	0	0

The recovery was calculated excluding the herbicide found in methanol after column washing; n.d., not detectable.

(up to 95%) while the less similar MCPA and mecoprop show a good recovery, even if not quantitative. The minor loss of MCPA and mecoprop in the washing step could be directly related to the low retention factors observed for these herbicides when eluted on the HPLC column packed with the imprinted polymer. It is remarkable that halogenophenoxy acid herbicides with low retention factors show themselves to be efficiently retained on the MISPE, while atrazine and bentazone (two herbicides whose molecular structure is completely different from the template) are not recognized by the imprinted polymer being washed away with the first milliliter of methanol.

3.6. MISPE of real samples

In Europe, the permitted concentration levels of herbicides in water are in the ng/ml range. Thus, to demonstrate the validity of the MISPE approach to the preconcentration of environmental contaminants, we spiked the water samples to a final concentration of 2–20 ng/ml with herbicides strongly retained by the imprinted polymer: 2,4,5-T, 2,4-D, fenoprop and dichlorprop. The loading of 100 ml of water sample, with an eluted volume of 2 ml (reduced to 200 μl after the evaporation step) results in a potential 500-fold concentration increase in the analytes for each extraction method considered. Presuming we have a quantitative recovery, it is a concentration of 1–10 μg/ml, a value that could be measured easily by capillary electrophoresis, using a suitable solution of cyclodextrines as running buffer to achieve complete resolution between the analytes.

From the results reported in Table 6, it could be observed that all the extraction techniques (liquid–liquid, reversed-phase and imprinted polymer-based) are able to extract the herbicides from river water, and that the reproducibility of the analytical data is not markedly different. In fact, the liquid–liquid extraction method is less reproducible and does not permit measuring fenoprop because of the presence of an interfering peak (Fig. 3), while the reversed-phase and MIP-based extraction are fully comparable in reproducibility, with quantitative recoveries of herbicide in all the situations considered.

Under these conditions the specificity of the method becomes a discriminating factor as both liquid–liquid and reversed-phase methods show many electrophoretic peaks (Fig. 3a,b) that cannot be ascribed to the herbicides but to numerous unknown interfering substances that are able to be more or less

Table 6
2,4,5-T, 2,4-D, fenoprop and dichlorprop recoveries at levels of 2, 5 and 20 μg/l using liquid–liquid, reversed-phase and molecular imprinted solid-phase extraction on 100 ml of river water

Herbicide		Recovery (%)		
		Liquid–liquid extraction	C_{18} reversed-phase extraction	MISPE
2,4,5-T	2 μg/l	97±39	88±21	89±17
	5 μg/l	91±36	105±17	93±11
	20 μg/l	89±41	93±10	94±5
2,4-D	2 μg/l	76±27	84±27	87±21
	5 μg/l	84±24	91±13	90±14
	20 μg/l	91±14	97±6	102±9
Fenoprop	2 μg/l	n.d	107±22	81±29
	5 μg/l	n.d.	84±29	91±19
	20 μg/l	n.d.	94±8	93±7
Dichlorprop	2 μg/l	66±33	93±25	114±19
	5 μg/l	79±24	96±13	89±13
	20 μg/l	71±26	103±8	90±6

The recoveries are calculated as the mean of five extraction experiments; n.d., not detectable.

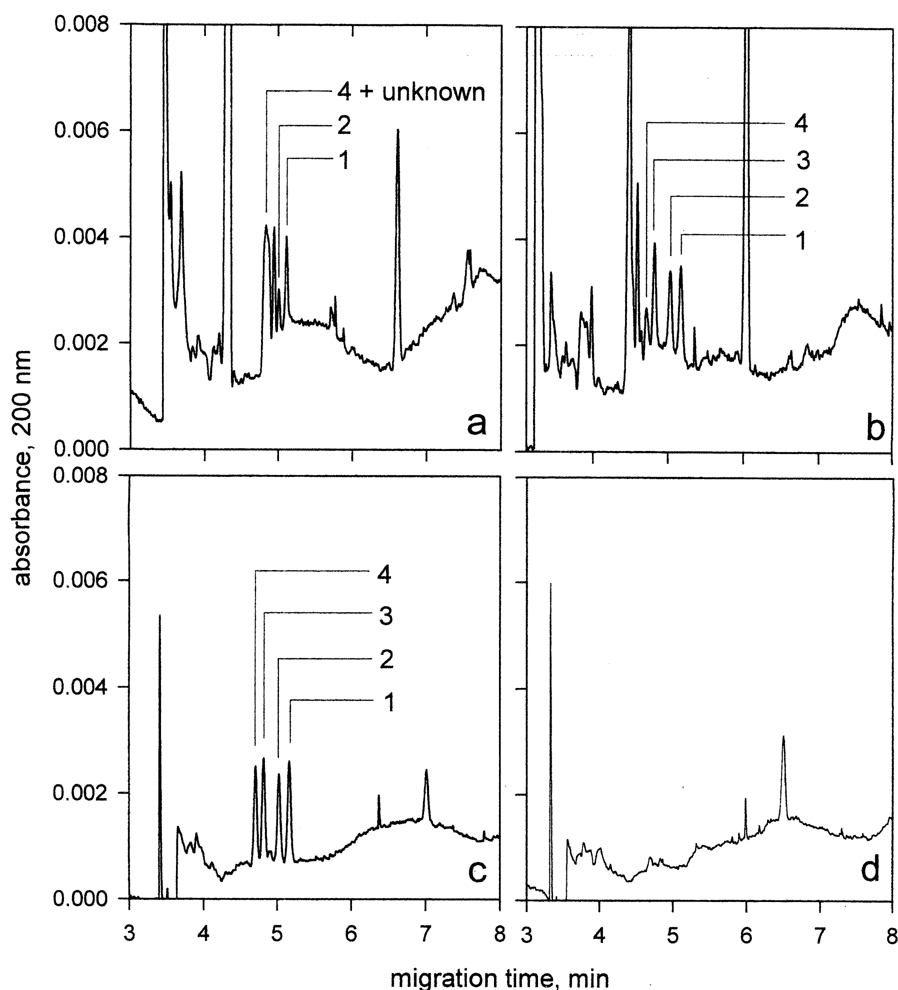


Fig. 3. Capillary electropherogram obtained after preconcentration of 100 ml of river water spiked at 5 μg/l with 2,4,5-T (1), 2,4-D (2), fenoprop (3) and dichlorprop (4) by liquid–liquid extraction (a), solid-phase extraction on a C_{18} column (b), solid-phase extraction on a MISPE column (c) and solid-phase extraction on a blank column (d). Separation condition described in the text.

included in the cyclodextrins of the running buffer. In contrast, the electropherograms corresponding to the MIP-based extraction (Fig. 3c) are simple, with the presence of very few interfering substances. It is significant that the selectivity should not be ascribed to the nature of the polymeric matrix itself but to the molecular recognition properties of the polymer, because (Fig. 3d) the extraction of a water sample performed on a blank column, not provided with specific binding sites for the template, causes an almost complete loss of analyte during the washing step.

4. Conclusions

The results obtained in this work confirm the possibility of obtaining halogenophenoxy acid-selective solid-phase extraction columns based on molecular imprinted polymers. The MISPE approach to the solid-phase extraction is comparable to the more traditional solid-phase extraction with C_{18} reversed-phase columns in terms of recovery percentage, but it seems to be superior in terms of sample clean-up where the interference by unknown substances is greatly detrimental for the quantification of the

analytes. In the near future more efforts will be made to apply these extraction techniques to samples of more critical complexity.

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