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Determination of phenoxyalkanoic acids and other herbicides at the ng/ml level in water by solid-phase extraction with poly(divinylbenzene-co-*N*-vinylpyrrolidone) sorbent and high-performance liquid chromatography–diode-array detection

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Abstract

A method for the determination of phenoxyalkanoic acids and other polar compounds in environmental water samples without pH adjustment before extraction has been developed. Recoveries were calculated from 500 ml of milliQ water spiked at the level of 0.5 ng/ml using solid-phase extraction (SPE) and HPLC–DAD. Different SPE materials (RP-C_{18}, ENV+, ENV+-C_8, SAX and Oasis HLB) were tested. After method optimization, 15 of the 16 compounds studied could be extracted with recoveries better than 70% on the most suitable copolymeric poly(divinylbenzene-co-N-vinylpyrrolidone) material (Oasis HLB cartridges).

Keywords: Phenoxyalkanoic acids; Poly(divinylbenzene-co-*N*-vinylpyrrolidone)

1. Introduction

Herbicides are typically determined in water samples following liquid–liquid extraction procedures, or by reversed-phase solid-phase extraction (RP-SPE). Most publications on this topic describe the use of modified silica gels or polymeric adsorbent-materials, especially RP-C_{18}, RP-C_8 or ENV+ sorbent (also known as PLRP-S or styrene–divinylbenzene material) [1–13]. In each case, the determination of several classes of herbicides having a wide range of chemical features requires distinct procedures. The major disadvantage is that the pH of the samples must be adjusted before extraction. For example, for the determination of phenoxyalcanoic acids, the pH of samples is adjusted to pH 2 with phosphoric acid [4–9], whereas for neutral or basic herbicides the pH of samples is adjusted to pH 6–7.

This work deals with the development of an analytical method using solid-phase extraction (SPE), followed by high-performance liquid chromatography (HPLC) with a diode array detector (DAD) [10–11], to determine simultaneously phenoxyalkanoic acids and other herbicides having a wide range of polarity at the ng/ml level in water without pH adjustment. To avoid pH adjustment and any other sample manipulation, we attempted to find suitable SPE-sorbents with similar behaviour and performances for both acidic, neutral and basic

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herbicides. In particular, we tested Oasis HLB (Hydrophilic–Lipophilic Balance) cartridges with other traditional SPE adsorbent-materials (RP-C_{18}, ENV+, ENV+-C_8 and SAX). This new reversed-phase for SPE is formed by the macroporous co-polymer poly(divinylbenzene-co-N-vinylpyrrolidone), which exhibits both hydrophilic and lipophilic retention characteristics. Two major features of this reversed-phase sorbent are the ability to remain wetted with water and to retain a wide spectrum of both polar and non-polar compounds. As we describe, these cartridges showed the best performances (efficiency of extraction and precision) for the determination of 16 contaminants having different chemical features.

The aim of our work was the study of environmental contamination in a naturally protected area originating from adjacent agricultural fields where cereals are cultivated. This protected area, called 'Bosco Tanali', is part of an ancient marsh that surrounded the Bientina Lake (Pisa, Italy), and it is today fed by some small rivers.

Among the potential contaminants, we addressed some polar molecules belonging to several classes of herbicides, such as triazines and particularly phenoxyalkanoic acids (Table 1).

These compounds are known to be the most commonly used products, and thus the need for a method for the routine monitoring of this area is clear. We further required a method that allowed for the assay of compounds at ng/ml levels, as a result of Italian law and EU directions imposing maximum residue levels of total herbicides ranging from 0.1 to 0.5 ng/ml in drinking water, 1–5 ng/ml in pretreatment potable water and 50–100 ng/ml in river water [14–15]. We chose the lower limit to ensure that the protected area is not affected by the surrounding agricultural lands.

We describe, firstly, the testing among various SPE sorbents and the determination of extracting recoveries of spiked milliQ water samples, and secondly, the application of the optimized method to environmental and drinking water samples coming from the area of interest and from other rivers or lakes monitored.

Table 1
List of searched herbicides contained in our work mixture solutions

Mixture 1	Mixture 2	Mixture 3
Dicamba	2,4-D	Desetil-atrazine
MCPA	Dichlorprop	Deisopropil-atrazine
2,4,5-T	2,4-DB	Bromacil
MCPP	Acifluorfen	Bentazone
MCPB	Oxyfluorfen	
Silvex	Chloridazon	

2. Experimental

2.1. Reagents and materials

2.1.1. Solvents

Pesticide grade ethyl acetate and cyclohexane were obtained from Merck (Darmstadt, Germany) and pesticide grade dichloromethane from Carlo Erba (Milan, Italy). HPLC grade methanol, ethanol (abs.) and acetonitrile were supplied by J.T. Baker (Deventer, Holland). Reagent grade trifluoroacetic acid (TFA) and glacial acetic acid were purchased from Carlo Erba (Milan, Italy), while reagent grade water was prepared by ultrafiltration with a milliQ system (Millipore, USA), (18.2 MΩ/cm). Phosphate buffer solution 5 *M* (pH 7) was obtained by reagent grade Na_2HPO_4 and KH_2PO_4 purchased from Carlo Erba (Milan, Italy). Phosphate buffer solution 0.05 *M* (pH 3) was obtained by reagent grade H_3PO_4 (85% w/v) and KH_2PO_4 purchased from Carlo Erba (Milan, Italy).

2.1.2. Pesticides

All pesticide materials and internal standards were of analytical purity and supplied by Lab. Ehrenstorfer (Augsburg, Germany). Stock solutions were prepared by dissolving approximately 10 mg of each compound in 10 ml of absolute ethanol. Three work mixture solutions were prepared at 10 μg/ml, diluting stock solutions with acetonitrile. The composition of these mixtures is specified in Table 1.

2.1.3. Internal standards

Two internal standard solutions were prepared in acetonitrile: 2-(4-chlorophenoxy)-2-methylpropionic acid (CMPA) at 10 μg/ml (IS1), and pentachlorophenol at 5 μg/ml (IS2).

IS1 was added to water samples before extraction, making it possible to control any analyte leak during this process. IS2 was instead added to residues just before injection, allowing for the monitoring of the extraction procedure by the peak area ratio of IS1 to IS2. This value should remain similar to the ratio obtained by the analysis of a solution containing the same concentrations of IS1 and IS2.

2.1.4. SPE cartridges

Solid-phase extraction was carried out with RP-C_{18} cartridges (500 mg–3 ml) obtained from J.T. Baker (Frankfurt, Germany); SAX cartridges (500 mg–3 ml) from SUPELCO (Milan, Italy); ENV+ cartridges (200 mg–6 ml) and ENV+ cartridges (200 mg)–C_8(500 mg)–6 ml, both from IST Isolute (Mid Glamorgan, UK); Oasis HLB cartridges (60 mg–3 ml) obtained from Waters Corporation (Milford, USA).

2.2. Apparatus

2.2.1. SPE workstation

The SPE procedure was carried out with a SPE vacuum manifold column processor from SUPELCO (Milan, Italy), connected to a vacuum pump. In this way, simultaneous extraction of twelve samples can be performed.

2.2.2. LC system and parameters

The liquid chromatography system used was the Hewlett Packard Mod. 1100 system, which was equipped with a degasser G1322A solvent delivery system, a quaternary pump QuatPump G1311A, an autosampler ALS G1313A, a column thermostat ColComp G1316A and a diode array detector DAD G1315A. The column used for these experiments was a Symmetry Shield RP_{18} endcapped 5 μm, 250×4.6 mm I.D. (from Waters); the flow-rate was 0.8 ml/min, the injected volume 10 μl (in acetonitrile); the temperature 40°C; the spectrum acquisition range was 210–350 nm with slit=2 nm; detection was carried out at 230 nm; band width, 12 nm.

The following conditions were used for the separation: the aqueous portion of the mobile phase (mobile phase A) was a 0.05 *M* H_3PO_4–KH_2PO_4 buffer solution at pH 3+0.01% (v/v) of TFA. The organic portion of the mobile phase was acetonitrile+0.01% (v/v) of TFA. A multistep gradient was employed to separate the analytes: it was 85% A initially, with a linear gradient to 55% A in 17 min, then linear to 35% A in 20 min and to 15% A in 8 min. It was held at 15% A for 5 min and finally in a minute it was carried again at initial conditions. Post-time for re-equilibration was 15 min.

2.3. SPE conditions

The cartridges were prewashed with 2×3 ml of ethyl acetate and 3 ml of methanol, then conditioned with 3 ml of methanol to solvate the functional groups of the sorbent and further with the sample matrix solvent (3 ml milliQ water). The cartridge was not permitted to run dry during the whole conditioning procedure. After sample loading was completed, elution of retained analytes was accomplished using 2×3 ml of ethyl acetate for C_{18}, C_8-ENV+ and ENV+ sorbents and 3 ml of ethyl acetate for Oasis HLB cartridges.

These SPE procedures were applied to all cartridge types, except the SAX ones. In this case the conditioning step was performed with 3 ml of methanol and then with 3 ml of a HPO_4^{2-}–$H_2PO_4^-$ buffer solution (0.05 *M*). The elution was carried out with a solution containing 20% of ethanol, 80% of ethyl acetate and 2% of acetic acid.

2.4. Procedure: sample preparation and SPE extraction

Five-hundred ml of milliQ water, containing 1% (v/v) of methanol [8], were spiked with work mixtures to obtain a concentration of 0.5 ng/ml, and added together with 25 μl of IS1.

For SPE–SAX extraction, 0.5 ml of a 5 *M* phosphate buffer solution were added to spiked samples.

Samples were then percolated through the preconditioned SPE cartridges at a flow-rate of about 5–10 ml/min. After drying, the cartridges were eluted applying a low vacuum (see Section 2.3) and the elute prepared as follows: eluted samples were evaporated under a gentle stream of nitrogen, and the residual dissolved in two portions of 1 ml and 0.5 ml of acetonitrile, respectively, and then transferred to

12×32 mm vials. These were again evaporated to dryness under a gentle stream of nitrogen, and the residue finally dissolved in 100 μl of IS2 solution, transferred to glass insert and autoinjected in HPLC–DAD. Two replicates of each injection were made.

The entire extraction procedure was repeated simultaneously three times for each group of herbicides, and for each tested RP adsorbent material. For the adsorbent that showed the best recoveries, the entire procedure was performed six times.

3. Results and discussion

To meet the objectives for the monitoring of acidic herbicides in water samples, a routine method has to be developed and evaluated. The aim of this study was to determine the SPE extraction procedure best suited for sample preparation and trace enrichment, importantly, without pH adjustment. This work was then extended to other active compounds of environmental interest that do not contain acidic protons, but have polar functional groups and a too high solubility in water to be well extracted with traditional RP materials.

3.1. Comparison of tested RP adsorbent materials

The results of our investigation show clearly that Oasis HLB cartridges [11] were superior to all other materials tested for our method without acidification of the sample. It was, therefore, selected for the screening of herbicides of interest in our water samples. Comparison between the several tested adsorbent materials is shown in Table 2. Oasis HLB cartridges are also successful in extracting active compounds belonging to other pesticides classes with different chemical features. For example, we found excellent recoveries of two metabolites of atrazine, desetil- and deisopropil-atrazine (Table 2). These are difficult to extract from water with RP-C_{18} owing to the presence of a free $-NH_2$ group in their structure [13]. Bromacil and Chloridazon also show a quantitative recovery with Oasis columns. In addition, Oasis HLB cartridges are water-wettable, and thus there is no need to ensure that it remains wet before loading the aqueous sample.

As predicted, most of the active compounds studied were found to give inadequate recoveries with RP-C_{18} adsorbent, and in fact, RP-C_{18} efficiency decreases as compound polarity increases. Several of the analytes, for example, MCPB, 2,4-DB, Oxyfluorfen and Bromacil, displayed good recoveries with RP-C_{18} (Table 2). Increasing aliphatic chain length results in an increase of recoveries of phenoxy-alkanoic acids from 2,4-D to 2,4-DB and from MCPA to MCPB. Although Oxyfluorfen and Bromacil belong to other pesticide classes (nitrodiphenyl-ether and uracil, respectively), they were used in this study because they have to be analysed by liquid chromatography. The polymeric material styrene–divinylbenzene (ENV+ cartridges) [12] was tested only with Mixture 1+Bentazone owing to the unsatisfactory results obtained with this mixture (only MCPB was recovered). The mixed material, ENV+-C_8, did not produce better results. Only five herbicides were found to be extracted (recovery >70%) from water with this material. We also carried out recovery experiments with SAX sorbent (strong anion exchanger) [7] for the adsorption of acidic compounds. To achieve optimum extraction conditions, the ion-exchange sorbent and the analyte should be oppositely charged. We therefore added a buffer to water samples to adjust the value to about pH 7, and to obtain the acidic compounds in the

Table 2
Percentage recoveries (n=3) of pesticides extracted from milliQ water spiked at the 0.5 ng/ml level with SPE using various adsorbent materials without pH adjustment

	C_{18}	ENV+-C_8	ENV+	Oasis
MCPB	93	84	83	89
Dicamba	2	0	0	17
MCPA	19	19	0	97
2,4,5-T	34	12	0	85
MCPP	9	14	0	82
Silvex	18	1	0	83
Bentazone	0	0	0	67
2,4-D	26	23	n.d.[a]	70
Dichlorprop	5	10	n.d.[a]	85
Acifluorfen	52	2	n.d.[a]	77
2,4-DB	91	81	n.d.[a]	108
Oxyfluorfen	90	74	n.d.[a]	104
Desetil-Atrazine	34	n.d.	n.d.[a]	108
Deisopropil-Atrazine	0	n.d.	n.d.[a]	91
Bromacil	72	75	n.d.[a]	99
Chloridazon	44	73	n.d.[a]	99

[a] n.d.=not determined.

proper form for retention on an anion-exchange column. For the same reason, for the elution of an acid from such a column, we had to convert the acid to its non-ionized form. In this case, no herbicides were extracted from water as a result of the competitive effects between buffer or matrix anions, and ionized acidic compounds for sorbent ionic functional groups.

3.2. Analytical performance

The calibration curves for quantitation were based on the analysis of solutions (in acetonitrile) containing 0.5, 1.0, 2.5 and 5.0 μg/ml of each analyte, corresponding to 0.1, 0.2, 0.5 and 1.0 ng/ml in 500 ml of water sample. IS1 and IS2 were added at 2.5 μg/ml and 5.0 μg/ml, respectively. Three injection replicates of each standard solution were made, and the calibration curves were obtained by plotting the peak-area ratio (PAR) versus analyte concentration. All analytes showed a good linearity in the range of concentration investigated (0.1–1.0 ng/ml), and R^2 values are included in the range 0.9814–0.9995.

The detection limit of compounds investigated was determined analyzing by HPLC–DAD system standard solutions at decreasing concentrations. From these chromatograms, we calculated for each analyte the S/N (signal/noise) ratio (height/height), and we considered a well-defined peak from bottom noise when its S/N ratio was ≥5. The method showed sensitivity limits for each herbicide comprised among 2–5 ng injected corresponding to 0.05–0.1 ng/ml in the considered volume sample.

The recoveries of analytes were calculated by a direct comparison between the results obtained processing spiked samples ($n=6$), where 25 μl of IS1 were added just before injection (ensuring no loss of IS1 during extraction procedure), and a standard solution containing the same level of concentration of analytes and IS1. For each analyte, the PAR (area of analyte peak versus area of IS1 peak) was calculated and percentage recovery was calculated as ($PAR_{sample}/PAR_{standard}\times 100$). Mean recoveries (R%) are listed in Table 3. Precision is given by the RSD% values, included in the range 6–32% ($n=6$).

Accuracy of the method was determined by replicate inter-day entire extraction procedures ($n=6$) of spiked milliQ water samples at the concentration of 0.5 ng/ml added of 25 μl of IS1 (as described in 2.4). For each analyte, the peak-area ratio (PAR, area of a compound peak versus area of IS1 peak) was calculated and the amount of each compound of interest determined from the calibration curve. Accuracy of method was calculated as the mean of measured concentrations versus nominal concentration (0.5 ng/ml).

Table 3
Mean recoveries (R%) and accuracy% with relative standard deviations ($n=6$) of herbicides extracted with Oasis HLB cartridges from spiked milliQ water samples at the level of 0.5 ng/ml

Compound	R%	RSD%	Accuracy%	RSD%
Dicamba	15	32	18	30
MCPA	95	15	78	10
2,4,5-T	86	9	96	13
MCPP	86	9	90	1
MCPB	92	8	82	4
Silvex	86	8	93	4
2,4-D	70	11	94	9
Dichlorprop	84	10	98	7
2,4-DB	101	14	83	10
Acifluorfen	71	24	82	24
Oxyfluorfen	91	21	94	18
Bentazone	72	15	93	18
Chloridazon	93	11	100	10
Bromacil	95	12	99	17
Deisopropil-atrazine	91	11	92	11
Desetil-atrazine	108	6	87	6

3.3. Applications

As application of the optimized method, we analysed water samples from the rivers of ‘Bosco Tanali’ to ascertain the presence of herbicides listed in Table 1.

We took water samples five times during a period of 18 months and the monitored rivers were three affluent streams and two artificial basins of stagnant water. These samples, before the assay, were filtered to eliminate suspended solid particles, then we measured a volume of 500 ml and added 25 μl of IS1 and 5 ml of methanol. The used extraction procedure is described in Section 2.4.

Fortunately, all water samples from the protected area did not contain herbicides in concentrations over the detection limit of the method. In contrast, MCPA was found in a sample from a river that wets fields near to another agricultural area. From a first screen-

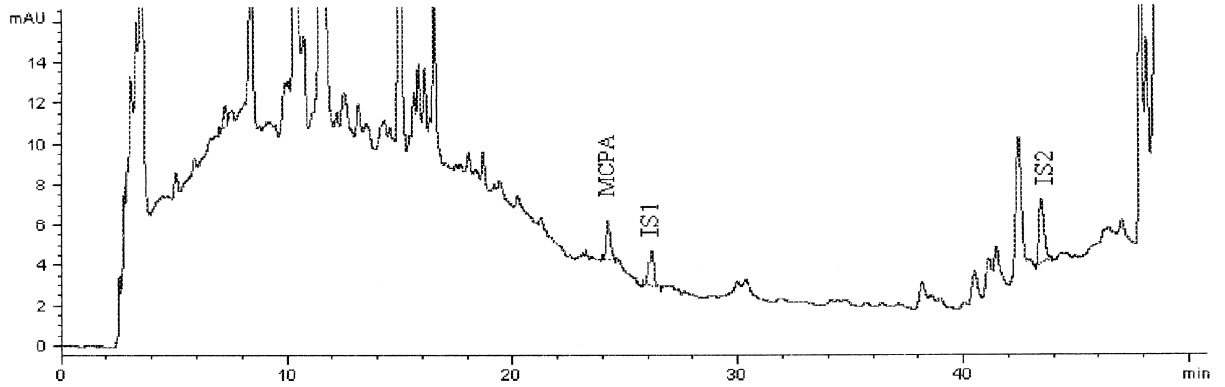

Fig. 1. Chromatogram of a river water sample obtained by the HPLC–DAD (230 nm) analysis after SPE–Oasis extraction procedure.

ing, this phenoxy acid resulted at a concentration that was over the highest level of the calibration curve. So we diluted the sample by a factor of 20 and repeated the extraction. The result was a concentration of 15 ng/ml. The HPLC–DAD chromatograms are showed in Figs. 1 and 2.

4. Conclusions

The SPE analytical method that we have developed, using Oasis HLB cartridges, presents several advantages:

- Simultaneous extraction of analytes with differing polarities and acidic-basic characteristics.
- Reduced time of analysis when compared with traditional SPE off-line methods where it is required that, to determine different classes of herbicides, the pH has to be adjusted before extraction of the sample.
- We are now extending the developed method to other classes of herbicides.
- With the high capacity of the Oasis HLB sorbent, less sorbent mass is required for cartridges. This results in a reduction in sample load time and elution volumes.

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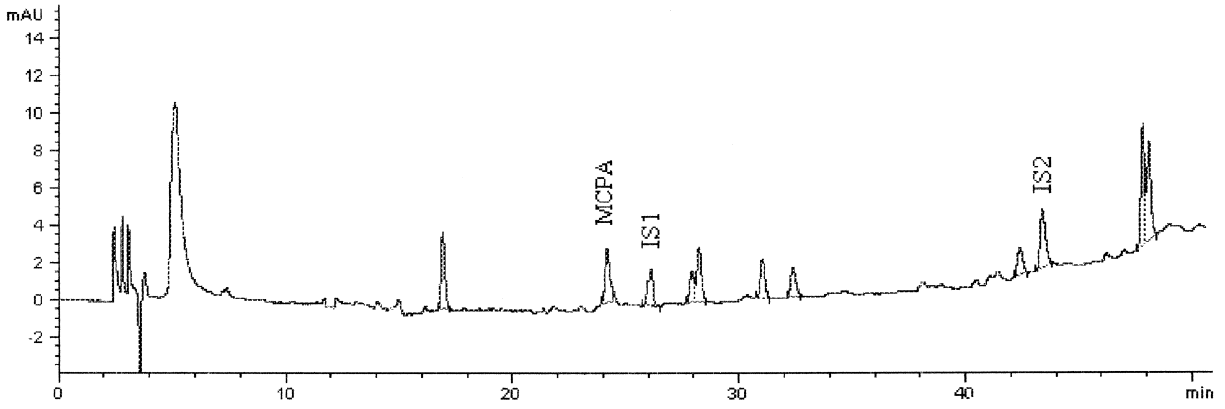

Fig. 2. Chromatogram of a standard solution, corresponding to the concentration of MCPA of 1 ng/ml in the sample, obtained by the HPLC–DAD (230 nm) analysis.

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