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The evaluation of different sorbents for the preconcentration of phenoxyacetic acid herbicides and their metabolites from soils

Sònia Moret, Juan M. Sánchez *, Victòria Salvadó, Manuela Hidalgo

Chemistry Department, University of Girona, Campus Montilivi s/n, 17071-Girona, Spain

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Abstract

A procedure using alkaline extraction, solid-phase extraction (SPE) and HPLC is developed to analyze the polar herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 4-chloro-2-methylphenoxyacetic acid (MCPA) together with their main metabolites in soils. An ion-pairing HPLC method is used for the determination as it permits the baseline separation of these highly polar herbicides and their main metabolites. The use of a highly cross-linked polystyrene-divinylbenzene sorbent (PS-DVB) gives the best results for the analysis of these compounds. This sorbent allows the direct preconcentration of the analytes at the high pH values obtained after quantitative alkaline extraction of the herbicides from soil samples. Different parameters are evaluated for the SPE preconcentration step. The high polarity of the main analytes of interest (2,4-D and MCPA) makes it necessary to work at low flow rates ($\leq$0.5 mL min^{-1}) in order for these compounds to be retained by the PS-DVB sorbent. A two stage desorption from the SPE sorbent is required to obtain the analytes in solvents that are appropriate for HPLC determination. A first desorption with a 50:50 methanol:water mixture elutes the most polar analytes (2,4-D, MCPA and 2CP). The second elution step with methanol permits the analysis of the other phenol derivatives. The humic and fulvic substances present in the soil are not efficiently retained by PS-DVB sorbents at alkaline pH's and so do not interfere in the analysis. This method has been successfully applied in the analysis of soil samples from a golf course treated with a commercial product containing esters of 2,4-D and MCPA as the active components.

Keywords: Phenoxyacetic acid herbicides; Solid-phase extraction; Soils; Polymeric sorbents

1. Introduction

Acidic herbicides, such as 2,4-dichlorophenoxyacetic acid (2,4-D) and 4-chloro-2-methylphenoxyacetic acid (MCPA), are widely used in the control of broad-leaved weeds and other vegetation. Once in the soil, the herbicides' behavior will be directly dependent on the soil properties and physicochemical properties of the compounds. 2,4-D and MCPA are degraded in soils to phenol derivatives, mainly 2-methylphenol (2-MP), 2-chlorophenol (2-CP), 4-chlorophenol (4-CP), 2,4-dichlorophenol (2,4-DCP), and 4-chloro-2-methylphenol (4-C-2-MP). The monitoring of these herbicides and their metabolites in soils is important to control their impact on the environment.

Several procedures for the extraction of these analytes from solid samples have been reported. Soxhlet extraction with different solvents is the oldest and most widespread method for the recovery of analytes from solid samples [1]. However, this method has many shortcomings such as the need to use large volumes of organic solvents and long analysis times. Another conventional method is solid–liquid extraction (SLE) [2–10]. SLE with organic solvents is also time consuming and requires large volumes of hazardous solvents. SLE with alkaline aqueous extraction, however, has been shown to be a simple and fast procedure for the extraction of phenoxyacetic acids [4,6–8,10] and phenols [11]. Recently, supercritical fluid extraction (SFE) [10,12–14], microwave-assisted solvent extraction (MASE) [10,15–17], and pressurized liquid extraction (PLE) [10,18–21] have demonstrated their potential as important alternatives to conventional methods, but the instruments involved have the drawback of being costly.

Chromatographic techniques are currently the most widely used in determining phenoxyacetic acids and phenolic derivatives. High performance liquid chromatography (HPLC) is preferred as it allows the direct analysis of these organic pollutants without previous derivatization. The trace analysis of compounds extracted from soil samples employing HPLC with UV

* Corresponding author. Tel.: +34 972418276; fax: +34 972418150.
E-mail address: juanma.sanchez@udg.es (J.M. Sánchez).

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detection, however, is hampered by the co-extracted humic and fulvic substances present in the soil, which cause a severe baseline deviation. This problem is solved by the performance of a clean-up and enrichment step before the trace level determination of the herbicides and their metabolites by HPLC.

Solid-phase extraction (SPE) by conventional bonded silica sorbents has been applied for the isolation and trace enrichment of organic contaminants from environmental samples before their analysis by chromatographic techniques [22]. Acidic herbicides are usually retained in their non-ionized form on bonded silica sorbents as phenoxyacetic acids are highly acidic (pK_a values are 3.07 and 2.64 for MCPA and 2,4-D, respectively [23]) and so it is necessary to work at pH < 2 in order to obtain the protonated form, which can be retained by the silica sorbents. Silica sorbents, however, are not stable at these acidic pH values and hence recoveries are small and non-reproducible.

Highly crosslinked polystyrene-divinylbenzene (PS-DVB) resins have proved to be more retentive than bonded silica sorbents, especially toward polar solutes [24–27]. This has been attributed to the aromatic, polymeric structure of the PS-DVB resins which can interact with aromatic analytes via π–π interactions [27,28]. Binding energies of π–π interactions with polymeric sorbents are higher than hydrophobic interactions [29], which are the main retention factors if C18 bonded silica sorbents are used. XAD-2 polystyrene resins have also been used for the retention of acidic herbicides, triazines, phenols and other aromatic compounds [8,30]. In recent years, new packing materials, such as Lichrolut EN [31] and Macronet Hypersol [32], and chemically modified polymeric resins with different functional groups (e.g. acetyl [33], hydroxymethyl [34], benzoyl [35], and *o*-carboxybenzoyl [36]) have been developed.

The aim of this study is to evaluate different sorbents for the determination of the phenoxyacetic acid herbicides 2,4-D and MCPA and their main metabolites from soil samples. The sorbents are tested to find which is the most appropriate for the clean-up and preconcentration of the analytes from the soils' alkaline extracts without the need for pH adjustment before the SPE procedure. Two styrene-divinylbenzene polymers (Bakerbond SDB-1, presented in a disposable cartridge, and XAD-2 resin) are evaluated and compared with a conventional C18 silica-bonded sorbent. After extraction, compounds are determined by ion-pairing HPLC (IP-HPLC) with UV detection. The IP-HPLC used here [37] is a variation of the method proposed by Geerdink at al. [38].

2. Experimental

2.1. Reagents and solutions

The herbicides, 2,4-dichlorophenoxyacetic acid (98.5%) and 4-chloro-2-methylphenoxyacetic acid (97.5%), and their phenol derivatives, 2-methylphenol (99.5%), 2-chlorophenol (99.5%), 4-chlorophenol (99.5%), 2,4-dichlorophenol (99.5%), and 4-chloro-2-methylphenol (99.5%), were used (Dr. Ehrenstorfer GmbH, Augsburg, Germany). Individual stock standard solutions (ca. 100 μg mL^{-1}) were prepared in methanol and stored in glass-stoppered bottles at 4 °C. Working mixtures were also prepared in methanol. Calibration standards used for the IP-HPLC method were prepared in methanol:water (50:50) for 2,4-D, MCPA, and 2CP, and in methanol for the other metabolites.

Acetonitrile and methanol were of analytical grade for pesticide residues (Carlo Erba, Milan, Italy). The HPLC mobile phase [37] consisted of acetonitrile:water (30:70, v/v) with 10 mM tetrabutyl ammonium hydroxide (TBA-OH) as the ion-pairing reagent (Fluka, Buchs, Switzerland). The pH of the mobile phase was adjusted to 7.2 with phosphoric acid (Panreac, Barcelona, Spain). This solution was filtered (0.45 μm particle size) and degassed prior to use.

The cartridges used for preconcentration were 3 mL disposable extraction syringes packed with either 200 mg of Bakerbond SDB-1 apolar copolymer (J.T. Baker, Deventer, The Netherlands) or 500 mg of AccuBOND II ODS-C18 sorbent (Agilent Technologies UK Ltd., West Lothian, UK). An Amberlite XAD-2 resin with a surface area of approximately 330 m^2 g^{-1} (Sigma, St. Louis, MO, USA) was also tested for the preconcentration step. Preliminary experiments showed that this resin was not adequately cleaned following the usual procedure for SPE cartridges. Therefore, the resin was cleaned by Soxhlet extraction using methanol, hexane, acetone, and methanol (12 h each) and kept under methanol until use [30]. A fixed bed-height of the XAD-2 resin was packed on a 10 mm i.d. glass column for the preconcentration and clean-up procedures.

2.2. Apparatus

The chromatographic experiments were performed with a Shimadzu liquid chromatograph (Kyoto, Japan) equipped with two pumps (LC-9A) and a UV–vis spectrophotometric detector (SPD-6AV). Samples were injected by means of a Rheodyne 7725i injector (Rohnert Park, CA, USA) with a 20 μL sample loop.

Ion-pairing HPLC (IP-HPLC) separations were carried out on a 20 cm × 0.46 cm i.d. column packed with a 5 μm Kromasil 100 C18 silica phase (Teknokroma, Barcelona, Spain). The flow rate was set at 1 mL min^{-1} and analyses were conducted at 25 ± 1 °C. Detection was performed at 230 nm for all the analytes. Fig. 1

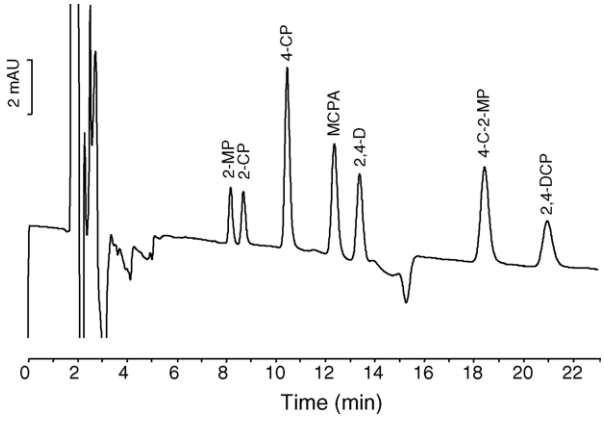

Fig. 1. Chromatogram obtained in the separation of a standard mixture containing the seven analytes evaluated in this study in methanol:water (1:1).

Table 1
Chemical and physical properties of the soil evaluated at surface level (0–10 cm depth)

Parameter	Value found
Organic matter (%)	1
Sand (%)	54
Mud (%)	34
Clay (%)	4
pH (1:1, in H_2O)	7.6
CEC (meq. g^{-1})	0.8
Conductivity ($\mu S\,cm^{-1}$)	710
Ca^{2+} ($g\,kg\,soil^{-1}$)	8.77
Mg^{2+} ($g\,kg\,soil^{-1}$)	1.13
Na^{+} ($g\,kg\,soil^{-1}$)	26.4
K^{+} ($g\,kg\,soil^{-1}$)	9.76
Natural density ($g\,cm^{-3}$)	1.3

CEC: cation exchange capacity.

shows a chromatogram obtained for a standard mixture containing the seven compounds of interest in methanol:water (1:1) using the IP-HPLC method described.

2.3. Sampling

Soil samples were drawn from a golf course in Pals (Girona, Spain). An area of 100 m^2 with a slope of <0.5% was chosen. This plot was divided into 10 sections for sampling purposes. The soil area was spiked by using ground spray application equipment loaded with a solution of 0.2 mL m^{-2} of Bi-Hedonal® (Bayer Hispania Industrial S.A., Barcelona, Spain), a commercial aqueous formulation of 2,4-D (27.5%, w/v) and MCPA (27.5%, w/v) as esters. This level of herbicides was chosen as it is the same as that which is regularly applied to the golf course for the control of broad-leaved weeds.

Samples were collected at three different depths (0–10 cm, 10–30 cm, and 30–60 cm), on different days after application, and at different locations within the plot in order to obtain an adequate number of representative samples. Soil samples were air-dried at room temperature for 3 days, sieved to a 2 mm size, and stored in amber glass bottles at −4 °C to prevent further degradation of the analytes.

The soil, which was of a mud-sand texture, was composed mainly of calcite and quartz with a low clay content (mean values for the different samples evaluated were 52% sand, 38% mud, and 6% clay). Table 1 shows the results obtained in the characterization of the surface level soil (0–10 cm).

2.4. Procedure for the evaluation of the solid-phase (alkaline spiking method)

Before evaluating the entire method for the determination of the analytes from soil samples, it was necessary to determine the clean-up/preconcentration performance of the different solid sorbents in order to find the most appropriate. Besides, it is necessary to take into account the effect of the soil matrix (e.g. organic matter content) on the overall performance of the method.

Soil samples (5 g), collected before the application of Bi-Hedonal®, were contacted with 10 mL of 0.01 M sodium hydroxide in a rotatory mixer at 30 rpm (Dinko, Barcelona, Spain) for 30 min at 25 ± 1 °C. Alkaline extracts were centrifuged at 2000 rpm for 20 min in a Mixtasel centrifuge (Selecta, Barcelona, Spain) to separate the soil supernatants and filtered through 0.45 μm filters (Whatman, Maidstone, UK). Filtered extracts containing the extracted organic matter were spiked with the analytes of interest at different concentration levels and the pH was adjusted to a predetermined value before passing these solutions through the solid sorbents. This process does not reproduce exactly the same matrix conditions as those of the real soil samples but it allows the evaluation of the effect of the soil matrix components on the recovery performance of the different sorbents tested and helps to determine the most appropriate conditions for the analysis of the compounds of interest.

Filtered-spiked extracts as well as posterior methanol or methanol:water elution solutions were passed through the sorbent cartridges by using a Minipuls 3 M312 (Gilson, Villiers Le Bel, France) peristaltic pump at different flows. The preconcentration and clean-up step procedure with the sorbents was as follows: (1) conditioning of the sorbent with 5 mL of methanol, (2) washing with 10 mL of milliQ water, (3) percolation of the extract, (4) washing of the cartridge with 5 mL of milliQ water, (5) drying the cartridge under vacuum for 30 s, (6) desorption of the metabolites and herbicides with the corresponding elution solution, and (7) direct injection of 20 μL of each of the elution solutions into the HPLC. Step (5) was omitted when XAD-2 resin was used as the sorbent.

2.5. Procedure for the validation of the entire method (spot spiking method)

For the validation of the method proposed in this study, 5 g soil samples drawn before the addition of Bi-Hedonal® were spiked with 1 mL of a methanol solution of the analytes. Alkaline extraction with 10 mL of 0.01 M NaOH in a rotary mixer at 30 rpm for 30 min (25 ± 1 °C) took place immediately after spiking the soil. The concentration of analytes in the spiked soil was in the range 1–100 $\mu g\,g^{-1}$. The other steps of the method were the same as described in the previous section.

3. Results and discussion

3.1. Alkaline extraction optimization

Previous studies [7,11] have demonstrated that the extraction of phenoxyacetic acids and chlorophenols from soils can be quantitatively performed with sodium hydroxide. A recent study [39] has shown recoveries of ≥90% for a broad range of chlorophenols from a certified soil using sodium hydroxide extraction. The use of alkaline extraction to extract polar compounds from soils is preferable to extraction with organic solvents as a large amount of both interfering and less-polar impurities remain in the soil sediment. Moreover, the procedure is easy to perform and does not require the use of hazardous organic solvents. The main drawback with alkaline extraction

Table 2
Analyte recoveries (%) and their uncertainties ($\pm t \times s/\sqrt{n}$, $\alpha = 0.05$) from soils obtained at different extractant concentrations

Compound	NaOH molarity			
	0.1 M	0.01 M	0.001 M	0.0001 M
MCPA	71 ± 8	76 ± 3	67 ± 10	76 ± 13
2,4-D	67 ± 10	76 ± 14	71 ± 11	76 ± 12
2-CP	85 ± 6	79 ± 5	74 ± 4	68 ± 8
2,4-DCP	89 ± 12	84 ± 10	79 ± 10	48 ± 15

Experimental: 5 g of soil spiked at 3 mg kg^{-1} with a single analyte and air-dried for 24 h, alkaline extraction with 10 mL extractant in a rotary mixer for 30 min, clean-up and preconcentration with the SDB-1 cartridge; elution with 5 mL methanol (three replicates each).

is that humic and fulvic substances present in the soil are co-extracted with the analytes.

Different molarities of alkaline extractant were tested to determine the most appropriate extraction conditions (Table 2). The two phenoxyacetic acid herbicides were evaluated together with 2-CP and 2,4-DCP as examples of phenolic metabolites. A variation of the spot spiking method described in Section 2 was used for these experiments: different 5 g portions of soil were each spiked with a single analyte at 3 mg kg^{-1} levels and air-dried for 24 h before extraction. Extraction was performed with 10 mL of sodium hydroxide solutions at the molarities indicated in Table 2. Alkaline extracts were neutralized and passed through an SDB-1 sorbent before being analyzed by the IP-HPLC method.

Preliminary kinetic studies showed that 30 min were enough to reach equilibrium in the alkaline extraction. Increasing the extraction times for periods of up to 6 h did not result in improved extraction of the analytes. The results show that the recovery of the phenoxyacetic acids (2,4-D and MCPA) was not affected by the molarity of the extractant used. This is due to the high polarity and low pK_a of these two compounds which results in a high affinity towards the aqueous extractant solution. These two facts also explain why the recoveries for these two compounds were at the same levels when the extraction step was performed with pure water.

The extraction of the chlorophenol derivatives, however, depended on the molarity of the extractant. The increase in the hydrophobicity and pK_a of these other analytes makes it necessary to use high concentrations of the extractant in order to have them in their ionic form, which permits extraction by the aqueous solution. The results show that there was a small variation in the recoveries obtained when the sodium hydroxide molarity decreased to 0.01 M, while lower molarities yielded a decrease in the recoveries. The use of water as the extractant resulted in no recoveries of the chlorophenol derivatives. Increasing the sodium hydroxide molarity, however, resulted in greater levels of co-extraction of the humic and fulvic acids present in soils. This led to lower clean-up efficiency of the SPE cartridges and an appreciable hump at the beginning of the chromatograms was observed after the clean-up step with the SDB-1 sorbent when soils were extracted with sodium hydroxide solutions at 0.1 M or higher molarities (Fig. 2).

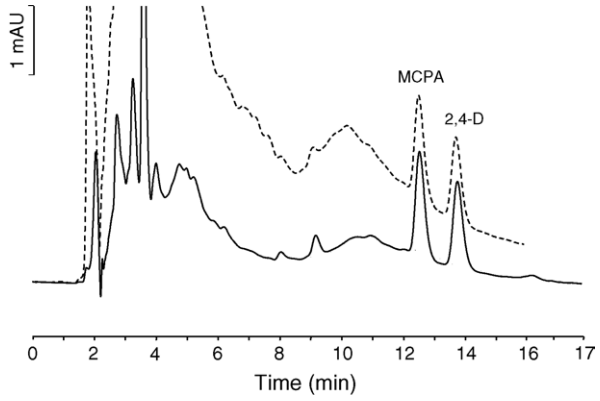

Fig. 2. Chromatograms obtained after SPE clean-up and preconcentration with the SDB-1 cartridge for a 5 g soil sample spiked with 2,4-D and MCPA. Extraction with 0.1 M (dashed line) and 0.01 M (solid line) sodium hydroxide. Experimental conditions as described in Section 3.1 (elution with 5 mL methanol:water, 50:50).

An 0.01 M sodium hydroxide solution was selected given that adequate recoveries were obtained for all the analytes and the level of co-extracted humic and fulvic substances was small enough to allow their adequate clean-up with the SPE cartridges.

3.2. Sorbent selection

Trace analysis of the acidic analytes extracted from soil samples by HPLC methodologies is affected by the co-extracted humic and fulvic substances causing a severe baseline deviation. For this reason it is necessary to clean-up the extracted sample before the HPLC analysis. SPE sorbents and polystyrene divinylbenzene resins provide a method allowing the simultaneous enrichment and clean-up of polar organic pollutants in water samples [22,27,28].

In order to select the best sorbent for the preconcentration of the phenoxyacetic acids and their metabolites from soil samples, we have evaluated two different commercially available SPE cartridges (a C18 silica sorbent and a polystyrene divinylbenzene sorbent) and a polystyrene divinylbenzene resin (XAD-2). The alkaline spiking procedure was used for these experiments. One of the main analytes of interest in this study (2,4-D) was chosen as an example for the evaluation of the cartridges because of its high polarity and low pK_a value. Once the best conditions for this analyte were found, all the other analytes of interest were evaluated in the same conditions to check the performance of the sorbent and conditions selected.

Phenoxyacetic acid herbicide solutions are usually adjusted to pH $\leq$ 2 in order to protonate the analytes and permit their quantitative retention in conventional silica sorbents [22,24]. As can be seen in Fig. 3, the percentage of retention for the 2,4-D herbicide in a conventional C18 sorbent was only quantitative at pH $\leq$ 3; higher pH values resulted in a sudden decrease in the retention of this herbicide to levels lower than 10%. This indicates that conventional silica sorbents can only be used if the sample is adequately acidified before SPE extraction. The need to adjust the sample pH, however, introduces a new step in

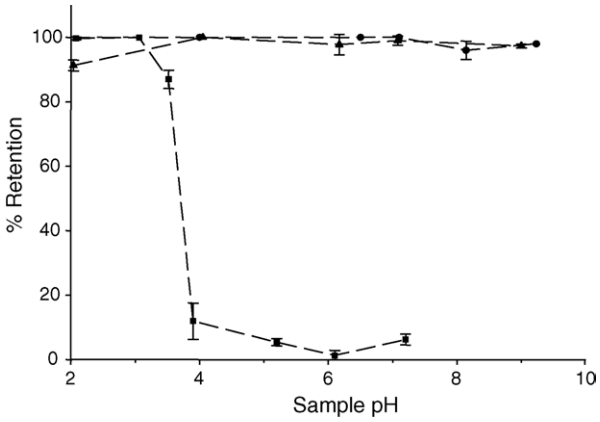

Fig. 3. Effect of the sample pH on the percentage of 2,4-D herbicide retained by different sorbents (■: ODS-C18 cartridge, ●: SDB-1 cartridge and ▲: XAD-2 resin). Experimental conditions were the alkaline spiking method described in Section 2 (three replicates each).

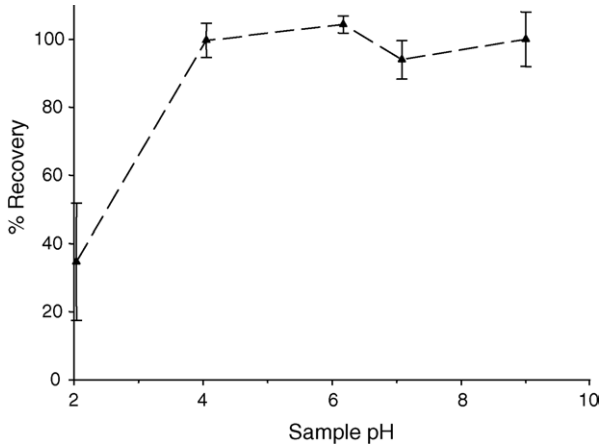

Fig. 4. Recovery percentages for 2,4-D herbicide at different sample pH's using the SDB-1 cartridge. Experimental conditions were the alkaline spiking method described in Section 2. Elution with 5 mL methanol:water, 50:50 (three replicates each).

the treatment that results in an increased variability in the final result and longer analysis times.

Polystyrene divinylbenzene sorbents (PS-DVB) are more retentive toward polar acidic herbicides than silica sorbents [22,28]. Moreover, PS-DVB sorbents have been used at neutral pH with recoveries for acidic herbicides similar to those obtained at acidic pH with silica sorbents, with no significant retention of the co-extracted humic and fulvic substances [28]. As can be seen in Fig. 3, the retention of 2,4-D was quantitative at pH values of up to 9.5 with the two type of polystyrene sorbents evaluated (pH values higher than 9.5 were not evaluated because the pH of the extracts obtained with 0.01 M NaOH were always lower than 10). This is explained by the fact that the primary sorption mechanism for PS-DVB sorbents is via π–π interactions with aromatic analytes [27,28], and it is not necessary for the protonated form of the analyte to be present to allow adsorption of the analyte. At pH 2, there was a decrease in the retention capacity for the XAD-2 resin, which can be explained by the fact that the humic and fulvic substances are not ionized at this pH and they can be better retained by the PS-DVB sorbents (this fact can be experimentally observed by the brown coloration that appears on the sorbents when samples are percolated at this pH). The high amount of humic and fulvic substances present in the soil extracts may result in saturation of sorbents with smaller capacities yielding lower retentions of the analytes of interest, as occurred with the XAD-2 resin because of its lower surface area (330 $m^2 g^{-1}$ for the XAD-2 resin and 933 $m^2 g^{-1}$ for the SDB-1 cartridge).

For the XAD-2 experiments, the polystyrene resin was located in a glass column with 10 mm i.d. at different bed-heights. The results obtained show that a 40 mm bed-height (1440–1450 mg of resin) was necessary to obtain the quantitative retentions of Fig. 3. Shorter bed-heights resulted in decreased retention capacities (89% for 20 mm bed-height and 50% for 10 mm). This is due to the smaller surface area of the XAD-2 resin that makes the use of a large amount of sorbent necessary to obtain similar retention values as those of the SDB-1 cartridge, which only contains 200 mg of sorbent.

The retention capacities of the sorbents evaluated show that the PS-DVB sorbents are more efficient for the quantitative retention of the acidic herbicide 2,4-D without the need to adjust the pH of the extracts before passing them through the sorbents. However, analytes can be strongly retained on the sorbent surface and can be difficult to elute. As the main objective of this study was to determine the content of the analytes in soil, the compounds have to be eluted quantitatively from the sorbent in order to be measured with the HPLC method. Therefore, determination of the recovery percentages of 2,4-D (calculated from the content in the spiked alkaline solution) is more useful than the retention parameter evaluated before. Fig. 4 shows the recovery percentages for 2,4-D obtained using the SDB-1 sorbent (5 mL of methanol were used as the elution solution). As can be seen, recoveries were quantitative in the 4–9.5 pH range. The large amount of co-retained humic and fulvic substances at pH 2 by the PS-DVB sorbents explains the smaller recoveries obtained and the high variability of the values at this pH. After elution of the analytes retained at pH 2, the sorbent still had a brown coloration, indicating that some humic and fulvic substances remained in the sorbent. Some portions of the analytes of interest can be strongly co-retained with these substances, which result in non-quantitative recoveries in these conditions and larger volumes of the elution solution being needed at pH 2. XAD-2 polystyrene divinylbenzene resin can be used effectively until neutral pH. However, the recovery of the analyte slowly decreased at higher pH values and about 80% of the initial analyte was recovered in the eluted solution at pH 8 and less than 70% at pH 9.5.

The results indicate that the SDB-1 sorbent is the best choice for the quantitative concentration and recovery of the 2,4-D herbicide when it is extracted from soil samples with 0.01 M sodium hydroxide. With this cartridge, it is not necessary to adjust the pH of the alkaline extract before the concentration and clean-up step. Table 3 shows the results obtained for the recoveries of the two phenoxyacetic acid herbicides (2,4-D and MCPA) and all their metabolites using the same procedure described for 2,4-D.

Table 3
Recoveries of the phenoxyacetic acid herbicides and metabolites (%) and their uncertainties ($\pm t \times s/\sqrt{n}$, $\alpha = 0.05$) using the SDB-1 cartridge

Analyte	Recovery (%)
MCPA	95 ± 5
2,4-D	96 ± 4
2-MP	101 ± 3
2-CP	93 ± 6
4-CP	97 ± 9
2,4-DCP	105 ± 6
4-C-2-MP	99 ± 11
2,4,5-TCP	101 ± 5

A single analyte was evaluated in each experiment. The experimental conditions were the alkaline spiking procedure described in Section 2 (0.01 M NaOH, extracts spiked at 1 mg L^{-1}, 0.5 mL min^{-1} sampling flow rate, elution with 5 mL methanol, three replicates each).

As can be seen, all the analytes were quantitatively recovered by using the SDB-1 cartridge when methanol was the desorption solvent.

Pichon et al. [28] suggested that polystyrene based sorbents do not retain humic and fulvic substances when the samples were percolated through the cartridge at neutral pH. Our results seem to suggest that this behavior takes place over a broad pH range (pH 4–9.5).

The volumetric flow rate during the adsorption step is an important parameter to be evaluated as the phenoxyacetic acid herbicides and their phenol derivatives are deprotonated at the alkaline pH of the extracts. Previous studies have demonstrated that acidic herbicides can be quantitatively retained by different cartridge sorbents at flow rates of up to 10 mL min^{-1} (e.g. Carbopack B and SAX [40], C18 bonded silica [25,41], XAD-2 resin [8], and polystyrene [28,31] cartridges). In most studies samples were acidified before loading the cartridge in order to protonate all the analytes. Pichon at al. [28] evaluated the extraction of some polar acidic herbicides with the SDB-1 cartridge at neutral pH and a retention flow rate of 10 mL min^{-1}, and obtained a quantitative recovery of the analytes. However, derivatives of phenoxyacetic acid, such as 2,4-D and MCPA, were not included. Luque-García et al. [15] found an optimum flow rate of between 0.5 and 0.7 mL min^{-1} for the retention of a group of phenoxyacetic acids (including 2,4-D) when using a mini-column filled with a C18-Hydra sorbent. Increased flow rate resulted in lower levels of retention associated to the retention kinetics.

We have evaluated the recoveries obtained for the two phenoxyacids (2,4-D and MCPA) and 4-CP, as an example of a chlorophenol, at different flow rates during the cartridge-loading step at alkaline pH. Recoveries for 4-CP were not affected by the loading flow rate in the cartridge and quantitative values were obtained at flow rates of up to 30 mL min^{-1}. However, recoveries of phenoxyacetic acid herbicides were highly affected by the loading flow rate. 2,4-D and MCPA can be quantitatively recovered at 0.5 mL min^{-1}, but their recoveries decreased at higher flow rates (about 80% recovery at 1.0 mL min^{-1} and 30% recovery at 30 mL min^{-1}). This can be explained by the higher polarity of these herbicides making it necessary to increase the contact time between the analyte and the polystyrene sorbent at this alkaline pH to allow the π–π interactions that permit the analytes to be retained by the sorbent. Increased flow rate results in shorter contact time and the retention kinetics do not allow the quantitative adsorption of the phenoxyacid analytes.

3.3. The evaluation of the desorption variables

3.3.1. Desorption solvent

The solvent used for the desorption of the analytes from the cartridge has to be compatible with the mobile phase used in the HPLC determination if no additional steps are to be introduced in the sample treatment. The limiting parameter for the IP-HPLC determination of the analytes was the separation of the 2,4-D and MCPA. The resolution (R_S) for this pair was found to be highly dependent on the composition of the solution to be injected. Baseline separation ($R_S = 2.0$) was obtained when 100% water solution was injected in the IP-HPLC method, but resolution decreased to 1.8 in 50:50 MeOH:water, and 1.4 in 60:40 MeOH:water. Increasing the methanol content of the solution resulted in an increased peak-width for both phenoxyacid compounds, especially 2,4-D. A methanol content of ≥70% resulted in a broad 2,4-D peak that made its separation from MCPA impossible for the quantitative analysis in mixtures. These results indicate that it is necessary to elute the samples containing 2,4-D and MCPA from the SDB-1 sorbent with a solution containing less than 60% methanol to achieve the quantitative determination of these two compounds.

The results obtained in the study of the volume needed for the quantitative elution of the analytes indicated that a first fraction, eluted with methanol:water (50:50), needed 5 mL for the quantitative recovery of 2,4-D and MCPA (recoveries were 106 ± 7 and 103 ± 3, respectively, $n = 3$), the most polar compounds. 2-CP and 4-CP, which are soluble in water, were also eluted under these conditions but recoveries were lower than 100%. 2-CP was eluted at >90% and 4-CP was only partially eluted (between 3 and 40% depending on the amount of analyte retained by the cartridge). The second fraction, eluted with methanol, needed 4 mL for the quantitative recovery of 2-MP, 4-C-2-MP, and 2,4-DCP, and the remaining 2-CP and 4-CP.

The main drawback of the proposed method is that a series of two elution solutions are needed to perform the analysis of all the compounds with the IP-HPLC detection method used in this study and the non-constant distribution of 4-CP between the two elution-solutions that results in an increased detection limit for this compound as it has to be analyzed in the two fractions. Fortunately, the sensitivity for 4-CP was larger than for the other analytes and its detection limit (Table 4) was still in the μg kg^{-1} range. This problem can be overcome by using a different HPLC method allowing the direct analysis of all the analytes in 100% methanol.

Fig. 5 shows the chromatograms obtained in the analysis of a soil spiked with the seven analytes at 1 mg kg^{-1} (spot spiking methodology as explained in Section 2 was used). Fig. 5a shows the chromatogram obtained for the first fraction eluted with 5 mL methanol:water (1:1). The dashed line corresponds to a blank from the same soil sample and treated in the same way as the

Table 4
Limits of detection (LOD) of the analytes in spiked soils and recoveries (%) obtained at different levels of spiked soil samples ($n = 3$)

Compound	Recovery (%)			LOD ($\mu g\,kg^{-1}$)
	1 mg kg^{-1}	10 mg kg^{-1}	100 mg kg^{-1}	
MCPA	107 (2.0)	105 (1.6)	104 (0.8)	5
2,4-D	104 (2.0)	102 (2.0)	100 (0.8)	10
2-CP	95 (3.6)	96 (2.4)	99 (0.8)	10
4-CP	95 (0.4)	95 (2.4)	97 (0.9)	5
2-MP	100 (2.0)	101 (2.4)	98 (1.6)	3
4-C-2-MP	105 (3.6)	102 (7.6)	104 (0.8)	3
2,4-DCP	100 (0.8)	103 (0.9)	101 (1.2)	10

Values in parentheses indicate the standard deviations obtained. Soil samples were spiked using the spot spiking method explained in Section 2.

spiked sample. As indicated before, 2,4-D, MCPA and 2-CP can be determined and quantified in this fraction. Fig. 5b corresponds to the second fraction eluted with 4 mL methanol. 2-MP, 4-C-2-MP and 2,4-DCP were quantitatively eluted with this fraction. Quantification of 4-CP was possible by determining the amount found in the first and second fractions.

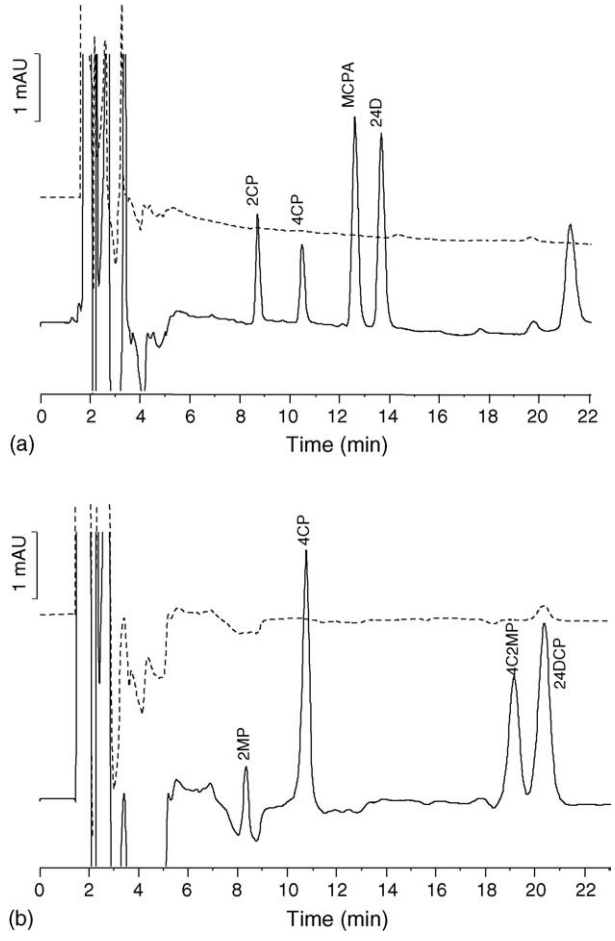

Fig. 5. Chromatograms obtained from a soil sample spiked at 1 mg kg^{-1} level: (a) first fraction eluted from the SDB-1 cartridge with 5 mL methanol:water (50:50) and (b) second fraction eluted with 4 mL methanol. Dashed lines correspond to blank soil samples analyzed following the same procedure as spiked samples (see Section 2 for spot spiking method).

3.4. Method validation

The spot spiking procedure was used in the validation of the entire method. The performance of the method was determined by processing the data obtained at three concentration levels of spiked soils (Table 4). Relative standard deviations in the recoveries of the analytes for three replicates were below 4% for practically all the analytes and concentrations evaluated. Analysis of the recovery data showed that there were no statistically significant differences between the values obtained at the three concentration levels for the analytes ($P = 0.74$).

Table 4 also shows the experimentally determined detection limits of the proposed method in the soil evaluated. These values were determined by spiking portions of soil at different levels. The detection limits indicated in Table 4 were those values with a signal/noise ratio ≥ 3 in the resulting chromatograms.

The EPA recommends method 8151A for the determination of 2,4-D and MCPA and other chlorinated acid herbicides in soil and waste matrices and this was used to validate the method proposed in our study. Soil samples were spiked as suggested by the EPA method and, from the two extraction procedures proposed, ultrasonic extraction with methylene chloride/acetone (1:1, v/v) was used. The results showed that the recoveries obtained with the method proposed (94% for MCPA and 98% for 2,4-D, $n = 4$) were greater ($P < 0.05$) than the results obtained with the EPA method (54% for MCPA and 56% for 2,4-D, $n = 3$). There was no loss of analytes with the proposed method.

3.5. Analysis of environmental samples

The method developed has been applied in the analysis of soil samples from a golf course treated with Bi-Hedonal®. Fig. 6 shows the chromatogram obtained for the first fraction (elution with methanol:water, 1:1) in the analysis of a sample taken 1 day after the application of the formulation at surface level (0–10 cm). As can be seen, the clean-up efficiency of the SDB-1 cartridge allowed the main portion of the humic and fulvic sub-

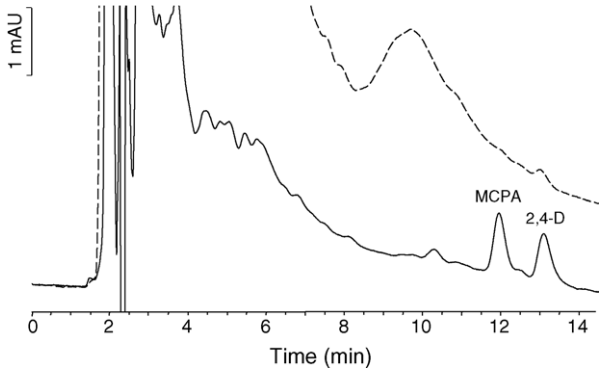

Fig. 6. Chromatograms obtained for the first fraction elution (50:50 methanol:water) for a surface level soil sample (0–10 cm depth) 1 day after the application of the commercial formulation. Solid line shows the chromatogram obtained after clean-up and preconcentration with the SDB-1 cartridge; dashed line shows the chromatogram corresponding to the same sample but before percolation through the SDB-1 cartridge.

Table 5
Concentrations found for the analytes in soils evaluated in this study

Sample no.	Days after application of the formulation	Sample depth (cm)	Soil			Analyte concentration ($\mu g\,kg^{-1}$ soil)						
			Organic matter (%)	pH	Conductivity ($\mu S\,cm^{-1}$)	MCPA	2,4-D	2-CP	2-MP	4-CP	4-C-2-MP	2,4-DCP
1	1	0–10	1.83	8.62	411	104	205	nd	nd	nd	nd	nd
2	3	0–10	1.71	8.80	441	73	112	nd	nd	nd	nd	nd
3	6	0–10	2.08	8.97	381	29	60	25	nd	nd	60	nd
4	14	0–10	2.12	8.92	292	12	d	34	47	nd	47	nd
5	37	10–30	1.00	8.90	190	nd	nd	nd	31	nd	nd	nd
6	22	30–60	0.26	8.36	692	nd	nd	d	d	nd	nd	nd

Experimental conditions: 5 g of soil, extraction with 15 mL 0.01 M NaOH. d: detected but below quantification limit and nd: not detected.

stances to be eliminated so making it possible to detect 2,4-D and MCPA.

Table 5 shows the concentrations found for all the analytes evaluated in this study for the different soil samples tested. The main analytes (2,4-D and MCPA) were found at their highest levels the first day after the application of the formulation. The concentration found for these compounds decreased slowly as they were degraded in soils [8] and after 14 days both compounds had practically disappeared. Some of the metabolites of the two phenoxyacetic acid herbicides started to appear after 6 days. A complete study of the soil herbicide content and their degradation is beyond the scope of this paper.

4. Conclusions

The SDB-1 cartridge, a polystyrene-divinylbenzene sorbent, performs outstandingly in the preconcentration and analysis of the highly polar herbicides 2,4-D and MCPA and their main metabolites from soil samples at trace levels. The results show that this cartridge can be used to retain these analytes at the high pH's resulting from alkaline extraction from soil samples without the need to adjust the pH of the extract as is necessary when conventional C18 cartridges are used. Humic and fulvic substances, which are present in large quantities in the extracts, are not efficiently retained by the cartridge when the samples are percolated at neutral or alkaline pH values. The high polarity of the 2,4-D and MCPA makes it necessary to use low flow rates ($\leq$0.5 mL min^{-1}) to assure the quantitative recovery of these two analytes given that they are weakly retained by the π–π interactions with the polymeric structure of the sorbents.

The main drawback of the method developed here is that the two phenoxyacetic acid herbicides, 2,4-D and MCPA, cannot be baseline separated with the IP-HPLC method used if they are eluted with methanol. However, they can be quantitatively desorbed with a 50:50 water:methanol mixture, which allows their separation and determination by IP-HPLC. This makes it necessary to perform two separate injections into the HPLC instrument to analyze both the herbicides and their metabolites.

The methodology developed has been applied in a preliminary study to determine the persistence of 2,4-D and MCPA in soils. The method has also been used to determine the phenol derivatives resulting from degradation by soil microorganisms. The results show that this is a simple, effective and fast method to analyze the polar herbicides 2,4-D and MCPA and their main metabolites in soil extracts.

Comparison of the results in Tables 2 and 4 confirms that analytes suffer ageing effect when they are allowed to remain in contact with the soil before the analysis (recoveries decreased from 100% to about 70% when the analytes were left in contact with the soil for 24 h before the alkaline extraction). This effect has been evaluated in our laboratory for some chlorophenols and it has been found that soil samples have to be conserved frozen in order to reduce this ageing effect [39].

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