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Fast, simple and efficient supramolecular solvent-based microextraction of mecoprop and dichlorprop in soils prior to their enantioselective determination by liquid chromatography–tandem mass spectrometry

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ABSTRACT

A simple, sensitive, rapid and economic method was developed for the quantification of enantiomers of chiral pesticides as mecoprop (MCPP) and dichlorprop (DCPP) in soil samples using supramolecular solvent-based microextraction (SUSME) combined with liquid chromatography coupled to mass spectrometry (LC–MS/MS). SUSME has been described for the extraction of chiral pesticides in water, but this is firstly applied to soil samples. MCPP and DCPP are herbicides widely used in agriculture that have two enantiomeric forms (R- and S-) differing in environmental fate and toxicity. Therefore, it is essential to have analytical methods for monitoring individual DCPP and MCPP enantiomers in environmental samples. MCPP and DCPP were extracted in a supramolecular solvent (SUPRAS) made up of dodecanoic acid aggregates, the extract was dried under a nitrogen stream, the two herbicides dissolved in acetate buffer and the aqueous extract directly injected in the LC–MS/MS system. The recoveries obtained were independent of soil composition and age of herbicide residues. The detection and quantitation limits of the developed method for the determination of R- and S-MCPP and R- and S-DCPP in soils were 0.03 and 0.1 ng g^{-1}, respectively, and the precision, expressed as relative standard deviation ($n=6$), for enantiomer concentrations of 5 and 100 ng g^{-1} were in the ranges 4.1–6.1% and 2.9–4.1%. Recoveries for soil samples spiked with enantiomer concentrations within the interval 5–180 ng g^{-1} and enantiomeric ratios (ERs) of 1, 3 and 9, ranged between 93 and 104% with standard deviations of the percent recovery varying between 0.3% and 6.0%. Because the SUPRAS can solubilize analytes through different type of interactions (dispersion, dipole–dipole and hydrogen bonds), it could be used to extract a great variety of pesticides (including both polar and non-polar) in soils.

1. Introduction

Enantiospecificity of chiral pollutants is a key factor to consider when assessing their health and environmental risks. Pollutant enantiomers significantly differ in their environmental fate and toxicological effects [1]. They can suffer exclusive or preferential degradation and/or interconversion in the environment [2,3], and their toxicities can differ up to more than 30-fold [1,4]. Consequently, it is essential to have analytical methods able to reliably monitor individual enantiomers of chiral pollutants in the different environmental compartments.

Among chiral pollutants, pesticides are of special concern because of their widespread use and their toxicity, mutagenicity, carcinogenicity and/or endocrine disruption activity. Mecoprop (MCPP) and dichlorprop (DCPP) are chiral herbicides frequently found in environmental waters and soils [5–8]. The half-life of these pesticides in soil is from a few days to several weeks, their concentrations ranging from micrograms to nanograms per gram of soil [8]. They have harmful effects on the biotic components of soils and reduce its fertility [9], and, owing to their high water solubility, they are amenable to transport to aquatic systems by rung-off and leaching of herbicide-treated soils, and therefore, there is a high risk of contaminating rivers, aquifers and other drinking water sources [8–10].

The presence of a chiral carbon atom in the aliphatic side chain of MCPP and DCPP gives two enantiomeric forms (R- and S-). Although the R-form is the unique and responsible for their herbicidal activity [11], both MCPP and DCPP are frequently produced and applied as racemic mixtures. Different degradation rates [2,8,12] and toxicities [13,14] for the R- and S-enantiomers of both herbicides have been reported.

Methods for determining MCPP and DCPP enantiomers in soils are based on gas chromatography–mass spectrometry (GC–MS) [15,16], liquid chromatography–ultraviolet detection (LC–UV) [17]

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and capillary electrophoresis–ultraviolet detection (CE–UV) [18]. A major handicap associated with the use of MS with LC or CE is the incompatibility of the mobile phases and chiral selectors commonly used in LC and CE, respectively, for chiral resolution of MCPP and DCPP. Analytes are extracted using methanol [13,27] or methanol/acetonitrile–water–acetic acid mixtures [16,18] with [17,18] or without [16,15] the assistance of ultrasounds, and extracts are cleaned-up by re-extraction in methylene chloride and concentrated by solvent evaporation. The extraction times and the volumes of organic solvent consumed for sample treatment vary within the intervals 1–2 h and 20–150 mL, respectively, and when GC–MS is used, diazomethane [15] or boron-trifluoride [16] are employed as derivatizing reagents. So, the development of simpler sample treatment methods to speed up sample throughput and save costs is of interest.

In this article, supramolecular solvents (SUPRASs) were firstly evaluated for the microextraction of chiral pollutants in soil samples prior to their enantiomer-specific quantitation by LC–MS. SUPRASs are nanostructured liquids made up of nanometer-sized aggregates produced through a self-assembly process [19]. They are produced from surfactant solutions by changing the temperature [20,21] or pH [22], or by addition of electrolytes [23], cosurfactants [24], amphiphilic counterions [25] or solvents [26,27]. Because of their high extraction efficiency and concentration capability, they have been largely used to extract organic compounds at low concentration levels in both liquid and solid samples [19]. In the environmental field, major applications focused on the analysis of aqueous samples [19], although methods for extracting pollutants such as polycyclic aromatic hydrocarbons (PAHs) [23,28] and surfactants [29] in soils [23,28], sediments [28] and sewage sludges [28,29] have also been reported. Recently, our group has described the use of SUPRAS for the extraction of chiral herbicides in environmental waters prior to LC–MS [30].

The SUPRAS used in this work to extract MCPP and DCPP enantiomers in soils consisted of three-dimensional aggregates of dodecanoic acid (DoA). The effect of experimental variables used for SUPRAS synthesis on the composition of the solvent was investigated, and the factors affecting the extraction efficiency of the target analytes and the cleanliness of extracts were optimized. The quality parameters of the developed method were assessed and both fresh and aged spiked samples of soils with variable organic matter content, pH and granulometric composition were analyzed.

2. Experimental

2.1. Chemicals

All chemical were of analytical reagent-grade and were used as supplied. Dodecanoic acid (DoA), racemic mecoprop (R/S-MCPP, 99.6% purity) and dichlorprop (R/S-DCPP, 99.9% purity), and the pure enantiomers R-MCPP (99.9% purity) and R-DCPP (99.9% purity) were purchased from Fluka (Buchs, Switzerland). Standard solutions (1 g L^{-1}) of R/S-MCPP and R/S-DCPP were prepared in methanol and stored under dark conditions at 4 °C. They were stable for at least 2 months. Deuterated R/S-MCPP (D_6, ring D_3, methyl D_3) and R/S-DCPP (D_6, ring D_3, 3,3,3-D_3), used as internal standards (ISs), were supplied by Dr Ehrenstorfer (Augsburg, Germany) as racemic solutions of 100 mg L^{-1} (D_6-R/S-MCPP in acetonitrile and D_6-R/S-DCPP in acetone; purity=98.5%). Working solutions containing mixtures of the target analytes (0.5 mg L^{-1} of each enantiomer) were prepared weekly in 100 mM acetate buffer at pH 5.0 and those containing mixtures of the ISs (0.1 mg L^{-1} of each enantiomer) were prepared in both methanol and 100 mM acetate buffer at pH 5.0. Methanolic IS solutions were used to spike soils before their analysis and the aqueous ones for preparing calibration standards. Tetrahydrofuran (THF), hydrochloric and acetic acid, ammonia, formic acid and sodium acetate were purchased from Panreac (Barcelona, Spain) and LC-grade methanol from Sigma-Aldrich (Steinheim, Germany). Ultra-high-quality water was obtained from a Milli-Q water purification system (Millipore, Madrid, Spain).

2.2. Apparatus

The LC–MS system used was a hybrid triple quadrupole/linear ion trap Applied Biosystems MSD Sciex 4000QTRAP (Applied Biosystems, Foster City, CA, USA) coupled to a liquid chromatograph Agilent HP 1200 Series (Agilent Technologies, Palo Alto, CA, USA) with a TurboIonSpray (TIS) interface. All data were acquired and processed using Analyst 1.5.1 Software. MCPP and DCPP enantiomers were separated on a chiral column Nucleodex α-PM (alpha cyclodextrin permethylated stationary-phase, 200 mm × 4.0 mm, 5 μm) from Macherey-Nagel (Düren, Germany). A magnetic stirrer Basicmagmix from Ovan (Barcelona, Spain) and a digitally regulated centrifuge Mixtasel equipped with an angle rotor 4 × 100 mL from JP-Selecta (Abrera, Spain) were used for SUPRAS production. A multi-position magnetic stirrer RO 10 power IKAMAG® from IKA®-Werke GmbH & Co. KG. (Staufen, Germany), a vortex-shaker REAX Top equipped with an attachment for 10 microtubes from Heidolph (Schwabach, Germany) and a high speed brushless centrifuge MPW-350R equipped with an angle rotor 36 × 2.2/1.5 mL from MPW Med-Instruments (Warschaw, Poland), were used for sample treatment.

2.3. Extraction efficiency studies

The effect of experimental variables on the efficiency of the microextraction of the racemic herbicides from soil was assessed by extracting 200–1200 mg of a sandy loam soil containing 3.8% organic matter (sample A in Table 1) spiked with 100 ng g^{-1} of racemic MCPP and DCPP, and determining the recoveries and the method quantitation limits for the R- and S-enantiomers of both pesticides. Method quantitation limits (MQLs) were calculated from the equation MQL=(100/R) SSR BSR IQL, where R is the recovery obtained in the SUPRAS-based microextraction, SSR the sample amount/SUPRAS volume ratio, BSR the acetate buffer volume/SUPRAS volume ratio used in the back-extraction step (see Section 2.4.3) and IQL the instrumental quantitation limit for the herbicides (0.05 ng mL^{-1}). The variables investigated were composition and volume of SUPRAS, sample amount, temperature, time for vortex-shake and centrifugation and rotation rate. The influence of experimental variables on the amount of humic substances extracted in the SUPRAS as well as the effect of the time of contact between the analytes and the soil samples on

Table 1
Physico-chemical properties of the soils tested.

Soil sample	Organic matter (%)	pH	Sand (%)	Silt (%)	Clay (%)	Textural class[a]
A	3.8	6.1	73	15	12	Sandy loam
B	1.3	7.9	58	13	29	Sandy clay loam
C	1.2	7.8	18	16	66	Clay
D	0.9	6.4	68	7	25	Sandy clay loam
E	0.8	7.9	10	44	46	Silty clay
F	0.5	7.0	80	6	14	Sandy loam

[a] USDA/FAO classification system [United States Department of Agriculture (USDA). Soil survey manual. U.S. Department. Agriculture Handbook No. 18 (1951) Washington, DC. Food and Agriculture Organization (FAO). Guidelines for soil description, 3rd edn. FAO/ISRIC (1990) Rome].

extraction yields was also studied. Experiments were made in triplicate.

2.4. Enantiomer-specific quantitation of MCPP and DCPP in soils

2.4.1. Collection, fortification and preservation of soil samples

Soil samples with variable organic matter content, pH and granulometric composition (Table 1) were taken from six different places in the province of Córdoba (Spain) from 0 to 20 cm in depth. After air-drying at room temperature, the samples were passed through a 2 mm sieve and stored at 4 °C under light protection until analysis.

Fortified samples were prepared by adding 50 μL per 100 mg of dried and sieved soil of a methanolic standard solution containing the racemic herbicides or a mixture of both racemic and pure R-herbicides. Enantiomer concentrations in samples were in the interval 0.1–900 ng g^{-1} and enantiomeric ratios (ER, defined as the molar ratio of R to S enantiomers) were 1, 3 or 9. After standing at room temperature for 24 h, fortified samples were analyzed (fresh spiked samples) or stored under dark conditions at 4 °C for 3 or 6 months (aged spiked samples). Analyses were made in triplicate.

2.4.2. Supramolecular solvent production

The following procedure, which permits to obtain a SUPRAS volume (~11 mL) able to treat 18 soil samples, was routinely followed. DoA (6.4 g) was dissolved in THF (12 mL) at room temperature in a 100 mL-glass centrifuge tube. Then, 68 mL of a 10 mM hydrochloric acid aqueous solution was added. After sealing the tube with parafilm to avoid THF evaporation, the mixture was magnetically stirred for 5 min at 900 rpm, during which the SUPRAS spontaneously formed into the bulk solution. Then, the suspension was centrifuged at 3500 rpm for 10 min to accelerate the separation of two liquid phases; namely the SUPRAS and a DoA-poor hydroorganic solution. Next, the SUPRAS, which is less dense than the hydroorganic solution, was withdrawn using a 20 mL-glass syringe, transferred to a hermetically closed storage glass vial to avoid THF losses and stored at 4 °C. Under these conditions the solvent produced was stable for at least month.

2.4.3. Microextraction in SUPRAS

About 800 mg of dry soil sample spiked with 120 μL of an IS standard solution containing 0.2 mg L^{-1} of racemic D_6-R/S-MCPP and D_6-R/S-DCPP (15 ng g^{-1} of each IS enantiomer), and 600 μL of SUPRAS were mixed in a 2 mL-microtube Safe-Lock from Eppendorf Ibérica (Madrid, Spain). Four glass balls (3 mm diameter) were introduced in the microtube to favor sample dispersion during extraction, which was made by vortexshaking at 2500 rpm for 5 min. Then, the mixture, thermostated at 20 °C, was centrifuged at 7000 rpm for 5 min. The volume of extract obtained after centrifugation was about 400 μL.

2.4.4. DoA removal

Aliquots of 200 μL of extracts were withdrawn using a microsyringe, transferred to 2 mL-microtubes and dried under nitrogen stream and magnetic stirring. The use of a multi-position magnetic stirrer and a glass manifold with six outlets (Pobel, Madrid, Spain) connected to the nitrogen supply permitted to simultaneously dry six extracts. After drying, 400 μL of 100 mM acetate buffer at pH 5.0 was added to the microtube, and the mixture was vortex-shaken for 5 min at 2500 rpm to guarantee the total dissolution of the target analytes. The aqueous extract containing the analytes was separated from solid DoA and water-insoluble sample matrix components by centrifugation at 15,000 rpm for 10 min. Finally, an aliquot of the supernatant was withdrawn with a microsyringe, filtered through a 0.5 μm PTFE filter, transferred to an autosampler vial and injected (40 μL) in the liquid chromatographic system.

2.4.5. Separation and quantitation of MCPP and DCPP enantiomers

The R- and S-enantiomers of both MCPP and DCPP were separated by chiral LC and quantified by tandem mass spectrometry (MS/MS). The mobile phase consisted of 65% methanol and 35% 100 mM formic acid/ammonium formate (pH 4.0). The flow rate was 0.5 mL min^{-1}, the stationary-phase column temperature was 25 °C and the injection volume 40 μL. The diversion valve was programmed to send the eluted components to the ionization source at run times in the interval 6.0–12.0 min. At times out of this interval, the mobile phase was sent to waste.

MCPP and DCPP enantiomers were identified and quantified in a mass spectrometer equipped with a TurbolonSpray (TIS, a variant of electrospray) source operating in the negative ion mode and a hybrid triple quadrupole/linear ion trap analyzer operating in the selected reaction monitoring (SRM) mode. The transitions (m/z) recorded were 213→141 (quantifier ion) and 213→71 (qualifier ion) for MCPP, and 233→161 (quantifier ion) and 233→125 (qualifier ion) for DCPP. Deuterated MCPP and DCPP used as ISs were monitored at the m/z 219→147 and 239→164 transitions, respectively. The TIS source and analyzer conditions were optimized to obtain the highest relative intensity. The TIS source values were as follows: curtain gas, 30 psi; nebulizer gas, 40 psi; turbo gas, 50 psi; temperature of the turbo gas, 600 °C; ion spray voltage, −2000 V; entrance potential, −10 V; and declustering potential, −50 V for the target analytes, and −60 V and −30 V for deuterated MCPP and DCPP, respectively. The analyzer settings were as follows: 1.0 unit resolution for the first and third quadrupoles; collision gas, 3.0×10^{-5} Torr; collision energy, −18, −16, −16, −40, −18 and −20 V; and collision cell exit potential, −11, −3, −7, −9, −9, and −1 V for the transitions 213→141, 213→71, 233→161, 233→125, 219→147 and 234→164, respectively. Calibration curves were constructed from standard solutions in 100 mM acetate buffer at pH 5.0 containing the target analytes over the ranges stated in Table 2 and constant concentrations of ISs (10 μg L^{-1} of each enantiomer). The concentration of the target analytes in the extract were calculated from calibration curves

Table 2
Figures of merit obtained for the determination of MCPP and DCPP enantiomers by LC(ESI)–QQQ-MS/MS.

Herbicide enantiomer	Retention time (min)	Calibration parameters				
		Linear concentration range (ng)	Slope ± s (ng^{-1})	Intercept ± s	r[b]	$S_{x/y}$[c]
R-MCPP	8.8	0.002[a] – 20	3.88 ± 0.03	0.004 ± 0.004	0.9998	0.18
S-MCPP	11.2	0.002[a] – 20	3.87 ± 0.03	0.005 ± 0.005	0.9997	0.23
R-DCPP	7.4	0.002[a] – 20	8.25 ± 0.07	0.01 ± 0.01	0.9996	0.55
S-DCPP	9.3	0.002[a] – 20	8.25 ± 0.07	0.01 ± 0.01	0.9997	0.49

[a] Instrumental quantitation limit calculated by using a signal-to-noise ratio of 10.
[b] Correlation coefficient, n=9.
[c] Standard error of the estimate.

obtained by plotting peak area ratios (A/A_{IS}; A=peak area of individual enantiomers and A_{IS}=peak area of the corresponding IS) versus the concentration of herbicide injected.

3. Results and discussion

3.1. Microextraction of MCPP and DCPP in soil

3.1.1. Synthesis, composition and binding capability of the DoA-based SUPRAS

The SUPRAS used to extract MCPP and DCPP in soils was synthesized by mixing DoA, THF and water at appropriate proportions (Fig. 1). The addition of water to solutions of DoA in THF caused the spontaneous formation of oily droplets that through a process of contact and adhesion form droplet conglomerates with a density lower than that of the solution in which they were generated. Droplet conglomerates separated from the bulk solution as an immiscible liquid (i.e. the SUPRAS). This coacervation process only occurred from the non-ionic form of DoA ($pKa=4.8\pm0.8$), and therefore, the pH of the aqueous solution employed in the synthesis should be equal to or lower than 4.

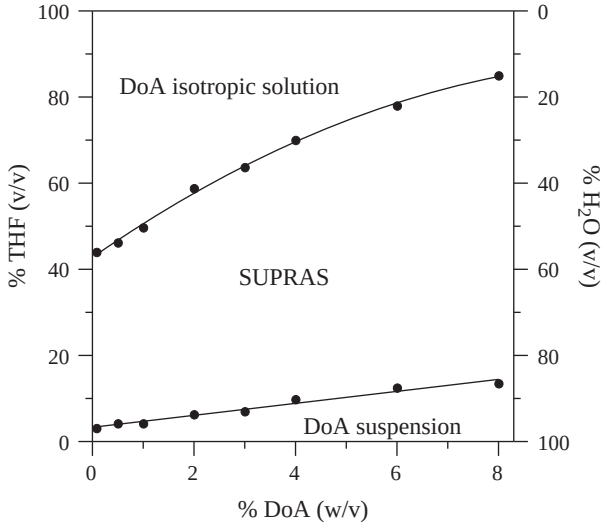

Fig. 1. Diagram of phase boundaries for ternary mixtures of dodecanoic acid, tetrahydrofuran and water obtained at pH=2 and room temperature.

Both the volume of SUPRAS generated (Fig. 2A) and the concentration of DoA in the SUPRAS (Fig. 2B) depend on the THF and DoA concentrations used to produce it. Fig. 2 shows the results obtained at DoA concentrations of 1% and 8%, w/v and THF percentages in the range 10–60% (v/v). The amount of THF and aqueous solution incorporated in the SUPRAS increased as the concentration of THF in the bulk solution did which resulted in a higher solvent volume (Fig. 2A) and a lower DoA content in the solvent (Fig. 2B). The volume of solvent also augmented with the amount of DoA employed in the synthesis (Fig. 2A), but the effect of this variable on the concentration of DoA in the SUPRAS was solely observed at THF percentages above 20% (v/v).

The pH used in the synthesis determined the concentration of hydrogen ions in the aqueous pool inside the reverse DoA aggregates making up the SUPRAS. No changes in solvent volume or biosurfactant content were observed by varying the pH within the interval 1–4.

The SUPRAS proposed in this study, can solubilize the target analytes through different types of interactions, namely hydrogen bonds between the oxy/carboxylic groups of MCPP and DCPP and the carboxylic groups of DoA and dispersion interactions between the hydrophobic moieties of the herbicides and the hydrocarbon chain of DoA. Taking into account the high number of solubilization sites in the solvent provided by the high concentration of amphiphile (0.1–0.65 g mL^{-1}, see Fig. 2B), it is reasonable to expect that the SUPRAS permits to effectively extract the target analytes from soil samples.

3.1.2. Factors affecting microextraction efficiency

The influence of SUPRAS composition on both the extraction efficiency of MCPP and DCPP in soil and cleanliness of the extracts was studied using 800 mg-soil samples and 600 μL of SUPRASs generated in solutions containing different DoA (1–8%, w/v) and THF (15–60%, v/v) concentrations, and variable pHs (1–4). Because MCPP and DCPP have similar polarities (log $K_{o/w,}$ 3.1 and 3.4 respectively) and hydrogen bonds formation capability [both contain the same number of hydrogen donors (1) and acceptors (3)], recoveries obtained for both herbicides were practically under the different conditions used for extraction. Although solvent composition did not influence the extraction yields obtained for the target analytes, it strongly affected the amount of humic substances extracted from soils. The higher the THF and water amounts in the solvent, the higher the organic matter amount in

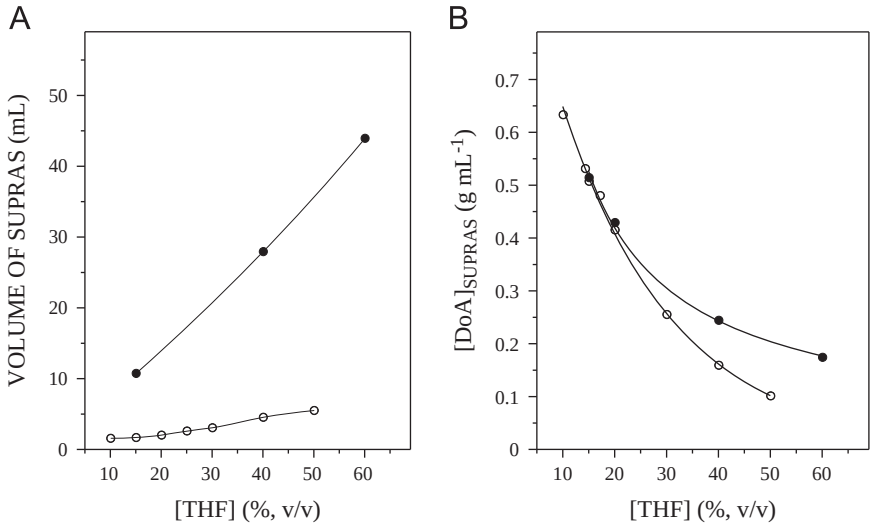

Fig. 2. Variation of (A) the volume of supramolecular solvent obtained and (B) the concentration of dodecanoic acid in the solvent as a function of the percentages of tetrahydrofuran and dodecanoic acid used to synthesize it. Percentages of dodecanoic acid: (○) 1.0% (w/v) and (•) 8.0% (w/v). pH=2. Total water+THF volume=80 mL.

Table 3
Mean recoveries and method quantitation limits obtained for MCPP and DCPP enantiomers as a function of the volume of SUPRAS and the amount of sample used for analysis.

Variable	SUPRAS volume/sample amount ratio (μL/mg)	Recovery ± s^a (%)				MQL (ng g^{-1})			
		R-MCPP	S-MCPP	R-DCPP	S-DCPP	R-MCPP	S-MCPP	R-DCPP	S-DCPP
SUPRAS volume (μL) (800 mg of sample)									
400	0.5	73 ± 1	69 ± 1	70 ± 1	72 ± 1	0.07	0.07	0.07	0.07
600	0.75	80 ± 2	78 ± 2	80 ± 2	81 ± 3	0.09	0.10	0.09	0.09
800	1	80 ± 1	81 ± 1	80 ± 1	81 ± 1	0.13	0.12	0.13	0.12
1000	1.25	81 ± 2	79 ± 1	81 ± 1	81 ± 2	0.15	0.16	0.15	0.15
1200	1.5	80 ± 3	80 ± 3	82 ± 2	83 ± 3	0.19	0.19	0.18	0.18
Sample amount (mg) (600 μL of SUPRAS)									
1200	0.5	69 ± 3	68 ± 3	66 ± 2	69 ± 1	0.07	0.07	0.08	0.07
800	0.75	80 ± 2	78 ± 2	80 ± 2	81 ± 3	0.09	0.10	0.09	0.09
600	1	78 ± 2	79 ± 3	77 ± 2	76 ± 2	0.13	0.13	0.13	0.13
400	1.5	80 ± 2	81 ± 2	77 ± 1	80 ± 1	0.19	0.19	0.19	0.19
200	3.0	80 ± 2	78 ± 1	79 ± 1	80 ± 1	0.38	0.38	0.38	0.38

[a] Standard deviation, $n=3$.

extracts. This behavior was similar to that previously reported for alkanol-based SUPRASs [27], which was explained on the basis of the different aqueous core sizes of the reverse aggregates making up the SUPRAS as a function of the THF/water ratio in the bulk solution where the SUPRAS was synthesized. The dodecanoic acid-based SUPRAS providing the extract with the lowest humic substance content was that produced from solutions containing 15% (v/v) THF, independent of the percentage of DoA and the pH employed in its synthesis; so, it was selected for further studies.

Table 3 shows the recoveries and MQLs obtained for the target analytes employing variable volumes of SUPRAS and sample amounts. Recoveries nearly 80% and independent of both solvent volume and sample amount were obtained at solvent volume/sample amount ratios equal to or higher than 0.75. To obtain maximum recoveries and minimum MQLs, it is recommended to extract 800 mg samples with 600 μL of SUPRAS.

Extractions at a controlled temperature were performed using a shaking incubator VorTemp™ 1550 (Labnet, Edison, NJ, USA) at 900 rpm (extraction time = 10 min). No effect of the temperature on recoveries was observed over the interval 25–60 °C, probably because these experimental conditions were not intensive enough to extract the herbicide fraction more strongly adsorbed on soil particles (bound residues [31]). At temperatures higher than the boiling point of THF (66 °C), DoA precipitated as a result of the reduction of the amount of organic solvent in the SUPRAS. To our knowledge, microwave assisted solvent extraction (MASE) at 80 °C has been the only approach reported to quantitatively extract phenoxyacid herbicides in soils [10], recoveries decreasing to 80–87% when conventional methods as repetitive or Soxhlet extraction were employed [16,32].

By using vortex shaking-assisted extraction (vibration motion = 2500 rpm), a time of 5 min was required to reach equilibrium conditions. Recoveries diminished at lower extraction times (e.g. they were 68 ± 3%, 67 ± 4%, 68 ± 4% and 65 ± 4% for R-MCPP, S-MCPP, R-DCPP and S-DCPP, respectively after 2 min of extraction). Effective separation of sample particles from SUPRAS extracts was reached after centrifugation at 7,000 rpm for 5 min.

Increasing the time of contact between the target herbicides and soil has been reported to decrease extraction efficiencies [10,32,33] owing to an increase in the pesticide–soil bond strength, this effect being more pronounced for organic matter rich soils [10,33]. For instance, extraction yields obtained for MCPP by MASE in samples stored 120 days at 4 °C decreased around 12% and 44% for soils with low (i.e. 0.3%) and high (i.e. 10.4%) organic matter content, respectively [33]. Also recoveries for DCPP in soils containing 3.5% of organic matter were reduced by 8% in 30 days [10]. To check whether ageing the fortified soils influenced extraction yields obtained by SUSME, soil samples containing 1.2% and 3.8% organic matter (samples C and A in Table 1, respectively) were spiked at three concentration levels (10, 100 and 500 ng g^{-1} of racemic MCPP and DCPP) and the fortified samples were stored in the refrigerator until analysis in order to inhibit soil microbial action and thus prevent herbicide degradation. Aliquots of these samples were analyzed 1, 90 and 180 days after the spiking following the procedure specified in Section 2.4.1. No effect of the time of contact on the extraction yields obtained was observed (e.g. the recoveries obtained by extracting the 3.8% organic matter soil spiked with 250 ng g^{-1} of each enantiomer after 1, 90 and 180 days were 80 ± 2, 77 ± 3 and 80 ± 1 for R-MCPP, 78 ± 2, 78 ± 1 and 79 ± 2 for S-MCPP, 80 ± 2, 78 ± 1 and 78 ± 3 for R-DCPP, and 81 ± 3, 78 ± 2 and 78 ± 2 for S-DCPP).

3.2. Analytical performance

3.2.1. Sample representativity

To evaluate the representativity of the amount of soil sample used for analysis, the variances obtained for the measurement of R- and S-MCPP and R- and S-DCPP in 800 mg soil subsamples fortified with 100 ng g^{-1} of each analyte were compared with those obtained from the measurement of 800 mg aliquots taken from a 50 g soil sample spiked at the same concentration level. No statistically significant differences between both variances were observed by applying a Fisher test [34]. The experimental F-values were in the interval 1.04–1.75 and were below the critical F-value (5.05, $n_1=n_2=6$, significant level = 0.05).

3.2.2. Linearity and sensitivity

Calibration curves for the target analytes were run using standard solutions in 100 mM acetate buffer at pH 5. Labeled herbicides [deuterated R/S-MCPP (D_6, ring D_3, methyl D_3) and R/S-DCPP (D_6, ring D_3, 3,3,3-D_3)] used as ISs were added to soil samples before extraction to control the performance of both analyte microextraction and MS detection. Calibration parameters and retention times obtained for R- and S-MCPP and R- and S-DCPP are shown in Table 2. The linear range for calibration curves was confirmed by visual inspection of the plot residuals versus analyte amount [35]. The number of positive residuals was approximately equal to that of negative ones and both positive

and negative residuals were randomly scattered around the average residual value.

The quantitation (MQLs) and detection (MDLs) limits of the method were estimated from calibration curves using peak areas 10 and 3 times higher than noise, respectively. The noise at the retention time of each analyte was measured from twelve independent complete analyses (experimental procedure in Section 2.4) of 800 mg-soil samples (six aliquots of sample A and six of sample C) containing no MCPP or DCPP at detectable concentration levels. No significant differences in the noise measured for the two types of soils were observed. The MQL obtained for the four enantiomers tested was 0.1 ng g^{-1} and their MDL 0.03 ng g^{-1}.

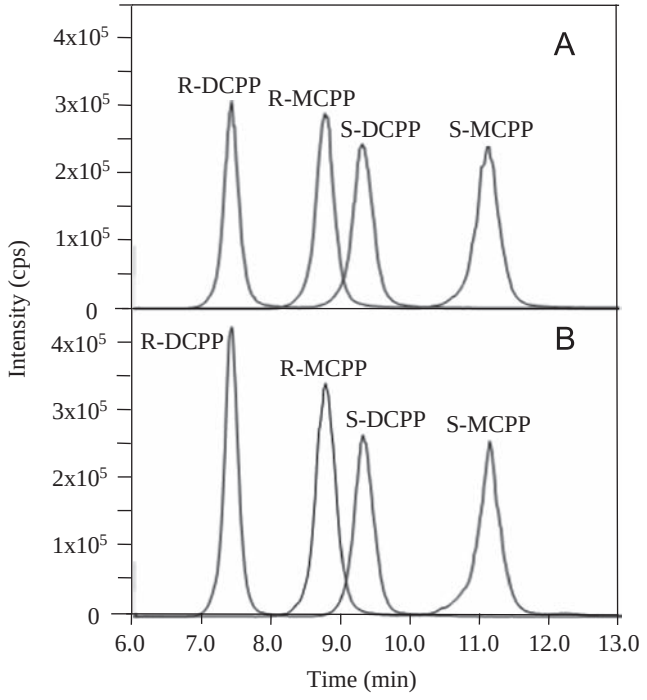

Fig. 3. LC–MS/MS selected ion chromatograms obtained from (A) a standard solution containing 50 μg L^{-1} of each enantiomer and (B) 800 mg of soil sample containing 3.8% organic matter (sample A in Table 1) and R/S–MCPP and R/S–DCPP at 100 ng g^{-1}.

3.2.3. Accuracy

The accuracy of the method was assessed by comparison of the slopes of the calibration curves obtained for the target enantiomers from standards in acetate buffer with those obtained from soil samples fortified with known amounts of racemic MCPP and DCPP (0.2–1500 ng g^{-1}), and run using the whole procedure. No statistically significant differences between the slopes obtained from standards and those obtained from the samples were observed by applying a Student's t test [36]. For example, the slopes and correlation coefficients ($n=9$) obtained from the soil sample A for R-MCPP, S-MCPP, R-DCPP and S-DCPP were 3.87 ± 0.03 ng^{-1}, 0.9997; 3.87 ± 0.05 ng^{-1}, 0.994; 8.18 ± 0.07 ng^{-1}, 0.9998 and 8.21 ± 0.07 ng^{-1}, 0.9994, respectively and those obtained for standard in acetate buffer were 3.88 ± 0.03 ng^{-1}; 3.87 ± 0.03 ng^{-1}; 8.25 ± 0.07 ng^{-1} and 8.25 ± 0.07 ng^{-1}. The experimental t-values were in the interval 0.07–0.64 and were below the critical t-value (2.98, significant level=0.01).

3.2.4. Precision

The precision was evaluated by analyzing independent soil samples spiked with racemic MCPP and DCPP at two concentration levels: 10 and 200 ng g^{-1} of each herbicide. The relative standard deviations ($n=6$) obtained varied within the intervals 4.1–6.1% and 2.9–4.1%.

3.3. Analysis of soil samples

The proposed method was used for determining MCPP and DCPP enantiomers in both non-spiked and spiked soil samples. Racemic MCPP and DCPP, and both racemic and pure R-herbicides were added to soil samples to obtain concentrations of the S- and R-enantiomers of the herbicides within the interval 5–100 ng g^{-1} and 5–180 ng g^{-1}, respectively, and ERs of 1, 3 and 9. No analytes were detected in the non-spiked samples. Results obtained for spiked samples are listed in Table 4. Recoveries ranged in the intervals 93–102% for R-MCPP, 94–104% for S-MCPP, 94–102 for R-DCPP and 94–103 for S-DCPP with standard deviations of the percent recovery varying between 0.3 and 6. No significant differences between calculated and determined ERs were observed. Fig. 3 shows the chromatograms obtained from a standard solution (A) and a fortified soil sample (B).

Table 4
Recovery and enantiomeric ratio (ER) of MCPP and DCPP from spiked soil samples.

Soil samples	Concentration added (ng g^{-1})				ER calculated		Recovery[a] ± s[b] (%)				ER determined d[a] ± s[b]	
	R- MCPP	S-MCPP	R-DCPP	S-DCPP	MCPP	DCPP	R-MCPP	S-MCPP	R-DCPP	S-DCPP	MCPP	DCPP
A	5	5	5	5	1	1	99 ± 5	97 ± 4	100 ± 6	100 ± 5	1.01 ± 0.07	1.00 ± 0.08
	100	100	100	100	1	1	99 ± 2	99 ± 4	96 ± 2	96 ± 3	1.00 ± 0.05	1.01 ± 0.05
B	5	5	5	5	1	1	99.0 ± 0.5	96 ± 2	102 ± 1	97 ± 6	1.03 ± 0.07	1.05 ± 0.06
	100	100	100	100	1	1	96 ± 1	94 ± 1	97 ± 1	99.0 ± 0.4	1.02 ± 0.02	1.00 ± 0.03
C	180	20	180	20	9	9	93.0 ± 0.3	95 ± 2	94 ± 3	94 ± 1	8.9 ± 0.2	9.0 ± 0.3
	150	50	150	50	3	3	99 ± 4	97 ± 2	95 ± 2	100 ± 5	3.1 ± 0.2	2.9 ± 0.2
	5	5	5	5	1	1	99 ± 2	96 ± 1	96 ± 4	96 ± 3	1.03 ± 0.03	1.00 ± 0.05
	100	100	100	100	1	1	98.9 ± 0.5	100 ± 3	97 ± 2	102 ± 1	0.98 ± 0.03	0.98 ± 0.04
D	5	5	5	5	1	1	97 ± 1	95 ± 1	100 ± 3	97.3 ± 0.8	1.02 ± 0.03	1.02 ± 0.03
	100	100	100	100	1	1	96 ± 4	104 ± 1	101 ± 3	99 ± 3	0.98 ± 0.02	1.03 ± 0.05
E	5	5	5	5	1	1	97 ± 3	95 ± 6	95 ± 5	94 ± 2	1.02 ± 0.07	1.01 ± 0.06
	100	100	100	100	1	1	98 ± 3	96 ± 2	94 ± 3	94.9 ± 0.5	1.01 ± 0.04	1.00 ± 0.03
F	5	5	5	5	1	1	102 ± 3	97 ± 3	98 ± 6	96 ± 3	1.05 ± 0.05	1.03 ± 0.06
	100	100	100	100	1	1	100 ± 6	101 ± 1	100 ± 6	103 ± 1	0.99 ± 0.06	0.98 ± 0.06

[a] Mean of three independent determinations.
[b] Standard deviation.

4. Conclusions

The combination of supramolecular solvent-based microextraction (SUSME) and LC–MS/MS is a valuable approach for determining enantiomers of MCPP and DCPP in soils. Because of its efficiency, simplicity, rapidity and low organic solvent consumption (0.65 μL of THF in the synthesis of the SUPRAS per soil sample analyzed) SUSME can be considered an outstanding alternative to the use of organic solvents in these type of applications. In contrast to previously reported methods [10,32,33], SUSME provides recoveries independent of the age of herbicide residues. Both the wide variety of interactions that SUPRASs can establish with analytes (i.e. hydrogen bonds, dipole–dipole, dispersion, etc) and the high concentration of amphiphiles they contain, make these solvents efficient extractants of contaminants from solid samples using very low volumes. So, they have the potential to extract trace amounts in soil without the need for solvent evaporation.

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