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Determination of the herbicide 4-chloro-2-methylphenoxyacetic acid and its main metabolite, 4-chloro-2-methylphenol in water and soil by liquid chromatography–electrospray tandem mass spectrometry

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Abstract

A rapid and sensitive LC–electrospray tandem mass spectrometry method has been developed for the quantitation of 4-chloro-2-methylphenoxyacetic acid (MCPA) and 4-chloro-2-methylphenol in both water and soil samples. Soil samples were extracted in alkaline media and cleaned-up by solid-phase extraction with C_{18} cartridges before LC–MS analysis. The selectivity and sensitivity offered by the triple quadrupole allowed the direct injection of the water samples rendering a sample throughput of around 100 samples per day, without any sample pretreatment, rendering for MCPA a limit of detection of 40 ng/l. In order to increase the method sensitivity, mainly for metabolite, a previous solid-phase extraction step was also performed. The method was validated by means of recovery experiments using fortified water and soil samples, obtaining satisfactory recoveries for both compounds in water and for MCPA in soil. The validated procedures can be used for the specific monitoring of residues of MCPA and its main metabolite in environmental samples, as ground and surface waters and soils, providing more selectivity and sensitivity than the current UV-based methodology. Besides, sample manipulation is greatly reduced in comparison to other GC–MS based methods which require a previous derivatization.

Keywords: Soil analysis; Water analysis; Environmental analysis; Pesticides; Alkylphenols; Phenoxyacetic acids

1. Introduction

Nowadays, contamination of ground water and soil with pesticides from agriculture is still a problem of major concern. Particularly in areas where the supply of drinking water is almost totally based on the use of ground water, pesticides have become an important part of ground water monitoring programmes.

Polar pesticides are the most likely to leach to ground water and, therefore, are the group of pesticides of primary interest in ground water monitoring. Within the different chemical compounds, phenoxyacid herbicides are widely applied. Particularly, 4-chloro-2-methylphenoxyacetic acid (MCPA) is widely used in Spain for weed control in citrus orchards may cause pollution of soils, ground and surface waters and potential risks to human health from direct exposure or through pesticide residues in drinking water. The availability of reliable data on the occurrence of MCPA residues in soil and water samples is of great importance for the proper assessment of these risks.

Analytical methods based on liquid chromato-

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graphy (LC) [1–5] are often preferred for the analysis of MCPA, more than gas chromatography (GC) based methods [6–8], as the sample pretreatment is greatly reduced. The traditional obstacle of this change, the lack of highly selective detectors in LC, is vanishing with the popularization of the atmospheric pressure interfaces (API) for direct coupling of LC to mass spectrometry (MS) [9].

LC–MS methods are very attractive because MS detection offers the possibility of achieving high sensitivity together selectivity. High sensitivity of the analytical method is required for its use in ground water monitoring, as a European Union (EU) directive [10] limits the content of individual pesticides in drinking water to 0.1 μg/l, which means that the methods applied should preferably have detection limits about one-fourth of this limit or lower, i.e., 0.025 μg/l or less. A high degree of selectivity is advantageous because it reduces the possibility of false positive findings. Besides, in this directive is also limited the content for the transformation products of parent pesticides. In relation to this, 4-chloro-2-methylphenol has been proposed as the major MCPA metabolite in the soil–water environment [11,12].

A number of different LC–MS interfaces, such as particle beam [13–15] and thermospray [16,17] have been used for the determination of MCPA; however, during the last few years, atmospheric pressure ionization (API) techniques, electrospray ionization (ESI) [18,19] and atmospheric pressure chemical ionization (APCI) [20–22] have become the more popular interfaces. Both of these techniques are soft ionization methods that predominantly give rise to the protonated $[M+H]^+$ or deprotonated $[M-H]^-$ molecular ions in positive- or negative-modes, respectively.

Acidic herbicides are due to their acidic properties most suited for negative ion mode LC–MS. Preliminary investigations carried out in our group demonstrated much better sensitivity for acidic herbicides using ESI than APCI [23].

An important aspect when performing residue analysis at the low concentrations relevant to soil and environmental waters is to assure a high degree of confidence in the identification of the compounds, in order to avoid false positives. The MS fragmentation pattern is a powerful tool for obtaining such confidence in compound identification. This fragmentation can be achieved using a single quadrupole by increasing the pre-analyzer extraction (skimmer cone) voltage [19,20,24]. However, by using tandem mass spectrometric detection, a more selective fragmentation of the initially formed deprotonated molecular ion is achieved by collision-induced dissociation (CID) in the collision cell between the first and second quadrupole [25].

While LC–MS–MS is the method of choice in quantitative bioanalysis, it is still used to only a very limited extent in environmental analysis [26]. Nevertheless, MS–MS for environmental analysis is gradually becoming more important, mainly in analytical strategies directed at rapid analysis [23,27,28].

Additionally, the volume required for sample pretreatment can be reduced from around 100 ml down to 10 ml or even less, as a result of the improved sensitivity of the LC–MS instrumentation. Another methodology is to perform on-line sample pretreatment by using the single short column (SSC) approach, applied for both preconcentration and minimum separation, using only 4 ml of water sample [29].

In this study we investigate the possibility of performing direct injection of different types of water samples by using LC–ESI-MS–MS for the rapid analysis of MCPA and its major metabolite, employing only 0.1 ml of water. Furthermore, another object of this study was to extend the scope of the application to soil analysis, in order to check the method robustness in more complex matrix samples.

2. Experimental

2.1. Reagents and chemicals

MCPA and 4-chloro-2-methylphenol reference standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany) and Sigma (St. Louis, MO, USA), respectively. HPLC-grade acetonitrile, acetone and methanol were purchased from Scharlab (Barcelona, Spain). LC-grade water was obtained by purifying demineralized water in a Nanopure II system (Barnstead, Newton, MA, USA). Analytical-grade hydrochloric acid (37%) and formic acid

(HCOOH, content >98%) were supplied by Merck (Darmstadt, Germany) and Fluka (Buchs, Switzerland), respectively.

Standard stock solutions were prepared dissolving 25 mg powder, accurately weighed, in 50 ml of HPLC-grade acetonitrile obtaining a final concentration of 500 μg/ml. For the LC–MS analysis, the stock solutions were mixed and diluted with LC-grade water. The concentration of the metabolite in all mixed standard solutions was 10-fold the MCPA concentration, due to smaller response in the case of the metabolite.

Disposable solid-phase extraction (SPE) cartridges containing 100 mg or 500 mg of C_{18}-bonded silica (40 μm) were obtained from J.T. Baker (Deventer, The Netherlands). A 24-place manifold of Varian (Harbor City, CA, USA) was used to perform SPE. Nylon filters of 0.45 μm and 25 mm diameter were obtained from MSI (Westboro, MA, USA).

2.2. Liquid chromatography

A high-performance liquid chromatography (HPLC) system Waters Alliance 2690 (Waters, Milford, MA, USA) was interfaced to a Quattro LC triple quadrupole mass spectrometer (Micromass, Manchester, UK). The LC separation was performed using a Nucleosil C_{18} 5 μm, 80×2 mm (Scharlab), at a flow-rate of 300 μl/min with mobile phases consisting of acetonitrile–0.01% aqueous HCOOH.

2.3. Mass spectrometry

A Quattro LC (quadrupole–hexapole–quadrupole) mass spectrometer with an orthogonal Z-spray-electrospray interface (Micromass) was used. Drying gas as well as nebulizing gas was nitrogen generated from pressurized air in a NG-7 nitrogen generator (Aquilo, Etten-Leur, The Netherlands). The nebulizer gas flow was set to approximately 80 l/h and the desolvation gas flow to 800–900 l/h. Infusion experiments were performed using a Model 11 single syringe pump (Harvard, Holliston, USA), directly connected to the interface.

For operation in the MS–MS mode, the collision gas was argon 99.995% (Carburos Metalicos, Valencia, Spain) with a pressure of $5 \cdot 10^{-4}$ mbar in the collision cell. Capillary voltages of 3 kV were used in the negative ionization mode. The interface temperature was set to 350°C and the source temperature to 120°C. Dwell times of 0.1 s/scan were chosen.

2.4. Sample procedure

2.4.1. Water analysis

2.4.1.1. Direct injection

Ground water and surface water samples were obtained from wells and lakes in the surrounding area (Castellón province, Spain). After filtration through 0.45-μm filters, in the case of surface water, 100 μl of sample was directly injected into the LC–MS–MS system for the determination of MCPA and metabolite.

2.4.1.2. Solid-phase extraction

A 50-ml volume of water sample was brought to pH 3 with formic acid and percolated through a preconditioned 100 mg C_{18} cartridge at a flow of approx. 4 ml/min. The SPE cartridges were preconditioned with 2 ml of methanol, 2 ml of water and finally 2 ml of acidified water (0.1% aqueous formic acid). After loading the sample, the cartridge was washed with 1 ml acidified water and dried by passing air for 30 min. The cartridge was then transferred to the top of a calibrated tube and, by means of slight over-pressure, 0.2 ml of methanol and 0.6 ml of water were passed sequentially through the cartridge and collected in the tube, adjusting the final volume to 1 ml with the acidified water. Finally, 100 μl of the 1-ml extract was injected into the LC–MS–MS system for the determination of MCPA and metabolite residues in water.

2.4.2. Soil analysis

Soil samples were obtained from two different agricultural areas in Spain. Soil A was collected from a citrus orchard in the Mediterranean area (province of Castellón) and soil B was from a greenhouse in the Jerte valley area, southwestern Spain (province of Cáceres). The organic matter of

these samples were found to be 0.8% (soil A) and 2.4% (soil B).

Air-dried soil samples were homogenized and 1.25 g subsamples were transferred to centrifuge tubes (50 ml). They were extracted by shaking with 0.5 *M* KOH (25 ml) on a mechanical shaker at 120 oscillations/min for 60 min, and then centrifuged (3000 rpm, for 15 min). Clear supernatant was transferred to a tube and dropwise neutralized with formic acid to a pH between 2 and 3, and the humic material was removed by centrifugation (3000 rpm, for 15 min). Then, 20 ml of the acidified clear supernatant was subjected to the clean-up step.

The acidified soil extract was percolated through a preconditioned 500 mg C_{18} cartridge at a flow of approx. 4 ml/min. The SPE cartridges were preconditioned with 6 ml of methanol, 6 ml of acetone, 6 ml of methanol and finally 6 ml of 0.1% aqueous formic acid. After loading the sample, the cartridge was washed with 3 ml 0.1% aqueous formic acid and dried by passing air for 30 min. Then, the cartridge was transferred to the top of a calibrated tube containing 0.5 ml of acetonitrile–0.1% aqueous formic acid (40:60) and, by means of slight overpressure, 2.5 ml of acetone was passed through the cartridge and collected in the tube. The acetone was evaporated by using a warm water-bath and a gentle stream of nitrogen and the final volume was adjusted to 1 ml by adding 0.1% aqueous formic acid. Finally, 100 μl of the extract was injected into the LC–MS–MS system for the determination of MCPA and metabolite residues in soil.

Fortification of air-dried soil samples was performed by using automatic pipet to deliver appropriate volumes of mixed standard, yielding fortification levels of 0.005–0.5 mg/kg. These samples were equilibrated under dark conditions, for 2 h, overnight or for 4 days, prior to extraction.

2.5. LC procedure

The mobile phase used for the isocratic analysis of both, water and soil extracts, was acetonitrile–0.01% aqueous HCOOH (35:65, v/v). In the gradient elution applied for water samples, the percentage of acetonitrile was changed linearly as follows: 0 min, 10%; 4 min, 30%; 8 min, 35%; 10 min, 40%; 11 min, 10%; 14 min, 10%.

2.6. Validation study

The precision (expressed as relative standard deviation, RSD) was evaluated within-day by determining MCPA (0.2 and 2 ng/ml) and 4-chloro-2-methylphenol (2 and 20 ng/ml) in two standard solutions ($n=8$). The calibration curve was obtained by analyzing standards solutions at eight concentrations between 0.2 and 100 ng/ml.

The recoveries for the direct determination were obtained by analyzing MCPA and 4-chloro-2-methylphenol in two ground water samples and one surface water sample spiked at two concentration levels (0.2 and 2 ng/ml for MCPA; 2 and 20 ng/ml for metabolite). In the case of applying the SPE preconcentration step the spiking level were 0.005 and 0.05 ng/ml for MCPA, and 0.05 and 0.5 ng/ml for metabolite. Recovery experiments were also carried out in two soil samples spìked at two concentration levels (0.005 and 0.05 mg/kg for MCPA, and 0.05 and 0.5 mg/kg for metabolite). In all cases, experiments were performed in quintuplicate ($n=5$).

The limit of detection (LOD), defined as the lowest concentration that the analytical process can reliably differentiate from background levels, was obtained when the signal was three times the background noise in the chromatogram at the lowest analyte concentration assayed.

Masslynx NT v 3.4 (Micromass) software was used to process the quantitative data obtained from calibration standards and from water and soil samples.

3. Results and discussion

3.1. Infusion experiments

The full-scan mass spectra and the MS–MS spectra of MCPA and 4-chloro-2-methylphenol are shown in Fig. 1. They were obtained from infusion of 5 μg/ml solutions (acetonitrile–water, 50:50, v/v) of each compound at a flow of 10 μl/min. As can be seen in Fig. 1, the electrospray ionization MS spectra of both analytes show a strong $[M-H]^-$ signal. The MS–MS spectrum of MCPA shows only one major fragment (m/z 141), and therefore high sensitivity

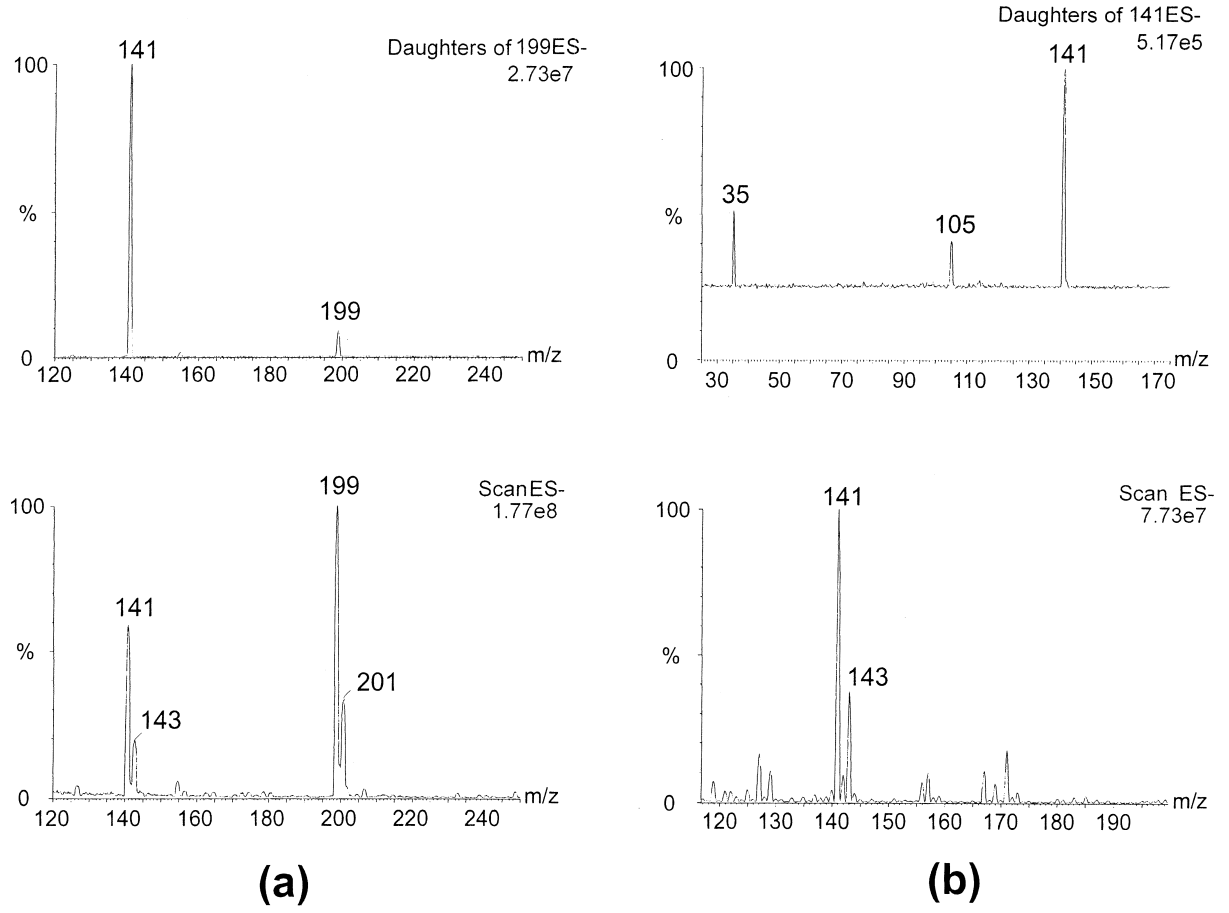

Fig. 1. The negative ion electrospray full scan mass spectra (bottom) and product ion spectra of pseudomolecular ion (top) of (a) MCPA and (b) 4-chloro-2-methylphenol.

can be expected from this optimal transmission efficiency. On the other hand, 4-chloro-2-methylphenol shows a minor fragment at m/z 35 that corresponds to the chloride ion (Fig. 1b). This transition is not very selective, and its low efficiency results on poor sensitivity. Although single ion monitoring (SIM) could also be used, Andreoli et al. have reported an approach for compounds without abundant fragmentation [30]. Precursor ion (m/z 141) is selected in the first quadrupole, and under low collision energy no fragmentation is observed, being also selected as the product ion in the last quadrupole. The collision energy selected should be optimized in order to break interferents but not the analyte ion; therefore cleaner chromatograms are obtained [30,31] in comparison to SIM acquisition.

3.2. LC optimization

Usually, the use of tandem mass spectrometry does not require chromatographic separation between analytes, as is very rare to find molecules which share the same unique transition. However, when dealing with pesticide metabolites, we have to take into account that the parent pesticide molecule can be fragmented in the sampling cone originating fragments with the same m/z that the metabolite produced in the environment, as the same bond cleavage is likely to occurr.

This is the case for MCPA and 4-chloro-2-methylphenol, as the deprotonated molecule of MCPA is fragmented in the sampling cone by generating 4-chloro-2-methylphenol. Thus, enough

Table 1
Mass spectrometry optimized parameters for the determination of MCPA and 4-chloro-2-methylphenol

MCPA (ESI)				4-Chloro-2-methylphenol (ESI)			
Precursor ion (m/z)	Cone voltage (V)	Collision energy (eV)	Product ion (m/z)	Precursor ion (m/z)	Cone voltage (V)	Collision energy (eV)	Product ion (m/z)
199	25	15	141	141	25	5	141
201	25	15	143	143	25	5	143

chromatographic resolution between these compounds is required to avoid the contribution to metabolite signal from the fragmentation in the source of the parent compound.

The chromatographic separation was easily achieved by using isocratic elution on a reversed-phase column with acetonitrile–0.01% aqueous HCOOH (35:65), however the effect of gradient elution on removal of salts and early-eluting polar interferences was also studied. In any case, the first part of the chromatogram was sent to waste by using the built-in divert valve in the mass spectrometer controlled by the Masslynx software.

Regarding the mass spectrometry acquisition, we checked selected ion recording (SIR) and also multiple reaction monitoring (MRM) for both compounds. A better signal-to-noise ratio was obtained for MCPA using MRM (S/N 20) than SIR (S/N 5), while similar data were achieved for the metabolite. Therefore, MRM recording was selected. Transitions, cone voltage and collision energies are shown in Table 1.

3.3. Analytical characteristics

Standard curves showed excellent linearity, with correlation coefficients of 0.9997 for MCPA (range 0.2–20 μg/l), and 0.9990 for 4-chloro-2-methylphenol (range 2–200 μg/l). Repeatability and reproducibility for standard solutions are reported in Table 2, using both isocratic and gradient elutions. The method was precise (RSD<9%) with instrumental limits of detection of 0.04 ng/ml for MCPA and 1 ng/ml for 4-chloro-2-methylphenol.

3.4. Water analysis

Typical chromatograms of standard solutions and water samples (blank and spiked at the lowest level

Table 2
Analytical characteristics of the developed LC–ESI-MS–MS method

	MCPA		4-Chloro-2-methylphenol	
Isocratic elution				
Linearity	Range=0.2–20 μg/l	r=0.9997	Range=2–200 μg/l	r=0.9990
Repeatability	0.2 μg/l	2 μg/l	2 μg/l	20 μg/l
(n=7, RSD, %)	8	4	10	8
Reproducibility	0.2 μg/l	2 μg/l	2 μg/l	20 μg/l
(2 days, n=6*, RSD, %)	9	5	11	9
Gradient elution				
Linearity	Range=0.2–20 μg/l	r=0.999	Range=2–200 μg/l	r=0.9993
Repeatability	0.2 μg/l	2 μg/l	2 μg/l	20 μg/l
(n=8, RSD, %)	2	1	7	6
Reproducibility	0.2 μg/l	2 μg/l	2 μg/l	20 μg/l
(2 days, n=6*, RSD, %)	3	3	9	8

*n=3, each day.

assayed) are shown in Fig. 2. They were obtained after direct injection of a ground water sample and by applying a gradient elution, and no interfering peaks were observed at the retention times of MCPA and 4-chloro-2-methylphenol. Chromatograms for MCPA (Fig. 2a) were cleaner than for 4-chloro-2-methylphenol (Fig. 2b) as we measured a real MRM (199>141) while for the metabolite we performed a ''pseudoMRM'' as the precursor and product ion were the same (141>141). In spite of this, the metabolite chromatograms were acceptable at these low levels because the triple quadrupole acted as a filtering device. Thus, some isobaric interferences could be broken down in the collision cell, while the analyte ion remains intact. In this way, some interferences can be removed by appropriate optimization of the collision energy used.

In order to decrease the detection limits of the procedure, a simple and fast SPE step with C_{18} cartridges was performed. Typical chromatograms of standard solutions and water samples (blank and 5 ng/l spiked with MCPA and 50 ng/l spiked with 4-chloro-2-methylphenol) are shown in Fig. 3, and they were obtained after SPE and applying an isocratic elution. In this picture we can observe that the spiked water sample used was not a ''real'' blank, as MCPA was present at a level as low as 1 ng/l. These low levels can only be detected after the SPE step, as the direct injection of the same sample (Fig. 2) produced a blank chromatogram.

Precision and accuracy for water analysis after direct injection or SPE extraction are reported in Table 3. The method was found to be precise (RSD<7%) and accurate (between 96 and 107% recovery) after direct water injection when using gradient elution (conductivity of all water samples< 3500 μS/cm). If isocratic elution was performed, even using the divert valve, we observed worse figures for both recoveries (79–94%) and precision (RSD<11%). However, after applying the SPE

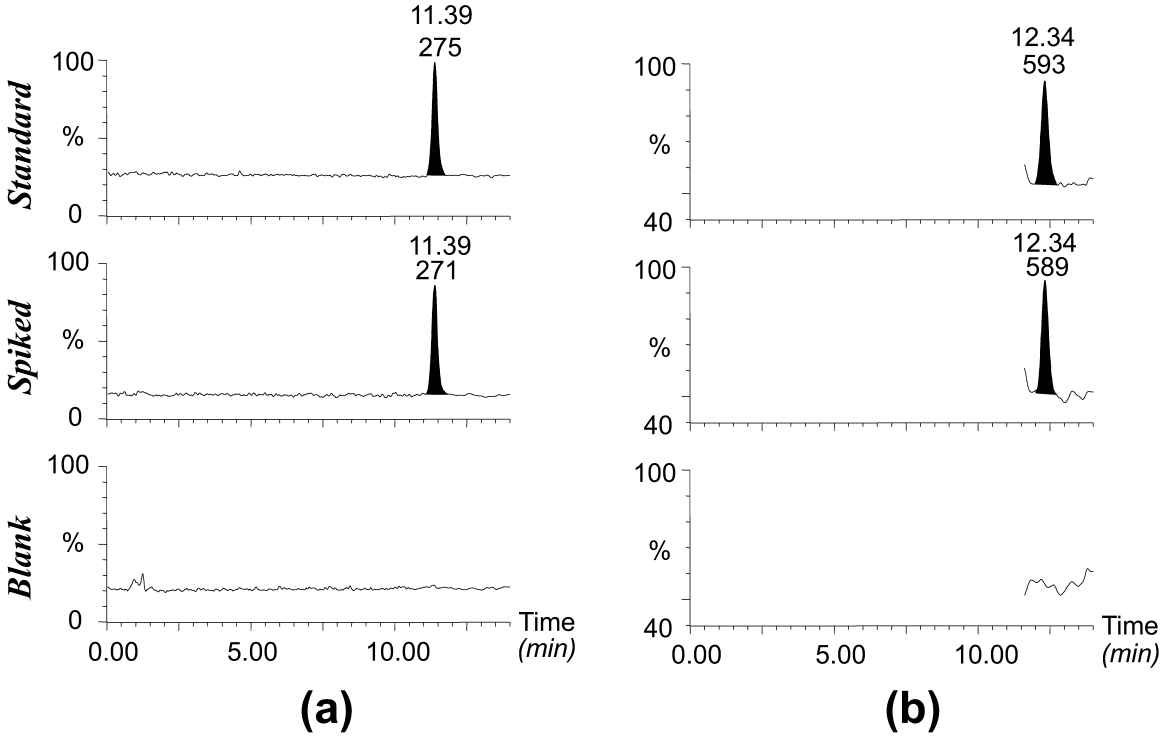

Fig. 2. LC–ESI-MS–MS chromatograms from direct injection of water samples (blank and spiked) and standards: (a) MCPA at 0.2 ng/ml and (b) 4-chloro-2-methylphenol at 2 ng/ml.

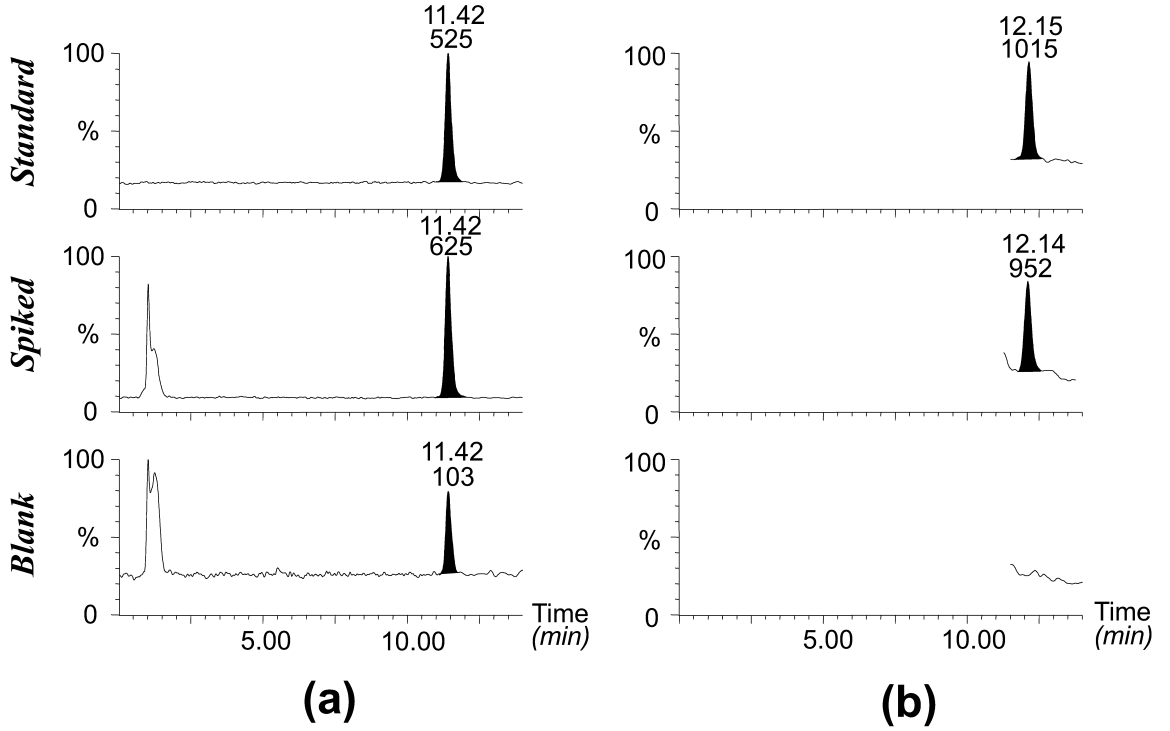

Fig. 3. LC–ESI-MS–MS chromatograms from SPE extracts of water samples (blank and spiked) and standards: (a) MCPA at 5 ng/l and (b) 4-chloro-2-methylphenol at 50 ng/l.

preconcentration step, the concurrent cleanup performed made that no differences in recoveries were observed between elution modes. As expected, precision was lower at the concentration levels in low-ng/l range, but the levels (RSD<17%) were still acceptable. The main adverse effect was observed on

Table 3
Validation study of the developed procedure for the determination of MCPA and 4-chloro-2-methylphenol in water samples

		Gradient elution								Isocratic elution							
		MCPA				4-Chloro-2-methylphenol				MCPA				4-Chloro-2-methylphenol			
		0.2 μg/l	2 μg/l	5 ng/l	50 ng/l	2 μg/l	20 μg/l	50 ng/l	500 ng/l	0.2 μg/l	2 μg/l	5 ng/l	50 ng/l	0.2 μg/l	2 μg/l	50 ng/l	500 ng/l
Direct injection																	
Ground water	Sample 1	98[a] (4[b])	99 (2)			101 (4)	99 (2)			90 (7)	87 (6)			86 (16)	94 (8)		
(n=5)	Sample 2	96 (2)	97 (5)			104 (3)	96 (3)			79 (9)	87 (2)			–	93 (11)		
Surface water (n=5)	Sample 1	107 (7)	96 (2)			–	98 (8)			82 (9)	85 (5)			–	84 (3)		
After SPE step																	
Ground water	Sample 1			106 (12)	95 (3)			94 (5)	99 (3)			108 (13)	104 (8)			95 (5)	97 (17)
(n=5)	Sample 2			108 (11)	97 (2)			100 (6)	100 (8)			105 (10)	93 (6)			92 (2)	91 (5)
Surface water (n=5)	Sample 1			63 (10)	66 (10)			83 (5)	78 (9)			71 (12)	77 (10)			82 (14)	85 (3)

[a] Recovery (%).
[b] RSD (%).

the MCPA recovery for surface water samples (65–71%). This was related to a higher level of interferences (even raised during the concentration step), which suppressed the ionization of analytes, specially for the first eluting analyte MCPA in the electrospray source, phenomena well documented in literature [32,33]. In order to overcome this problem, different possibilities could be chosen ranging from using an expensive stable isotope labeled MCPA, if commercially available, to preparing matrix-matched standards, if appropriate blank matrix samples are accessible. These solutions are not worth to assess in our case to obtain proper quantitation in surface samples at the very low level of 5 ng/l, when direct injection of surface water samples allowed us to reach limits of quantitation around 0.2 μg/l, low enough for these kinds of samples [34].

3.5. Soil analysis

In the case of soil samples, a previous extraction of analytes is mandatory. Considering the acidic behavior of both compounds, an alkaline media was selected, and strong (potassium hydroxide) and weak (ammonia) base solutions were assayed, following direct injection of the clear extract in the LC–MS–MS system. The results were not satisfactory in any case as recoveries ranged between 15 and 30% at the 0.05 mg/kg level. Therefore an additional clean-up step was necessary in order to decrease the co-extracted interferences as well as the high salt content due to the use of the strong base extractant selected (KOH), which affected the response of the electrospray source. A simple SPE with 500 mg C_{18} cartridges were selected.

The soil samples were fortified with analytes at 0.05 mg/kg and let stand overnight before extraction. Two soil/solvent ratios were used, low (1:2) and high (1:16), in order to check the effect on the analytes recoveries. Initially, the analytes were eluted from the SPE cartridges with 2.5 ml acetone; the solvent was evaporated to dryness under nitrogen stream and the residue dissolved in 1 ml acetonitrile–0.1% aqueous formic acid (30:70). Under these conditions, the results showed satisfactory recoveries for MCPA, both at low (82%) and high (86%) ratios, but in the case of the metabolite the recoveries were too low (24%), especially with a 1:2 soil/solvent ratio (7%).

In order to check if the low recoveries for the metabolite were related to the extraction process or to matrix suppression in the electrospray source, blank soil samples were extracted as before, and the final extracts were spiked with 4-chloro-2-methylphenol. In this case, satisfactory recoveries (100–102%) were found for both soil-to-solvent ratios. Therefore, there was no matrix effect on the phenol signal, and the low recoveries should be related to losses during the extraction and/or clean-up steps. Taking into account the volatility of phenols and assuming that some losses could happen during the evaporation step after the SPE cleanup, the process was slightly modified using a graduated glass tube containing 0.5 ml of acetonitrile–0.1% aqueous formic acid (60:40) to collect the acetone eluate, in order to minimize the phenol losses during the following evaporation under nitrogen. Data obtained showed that the recoveries for 4-chloro-2-methylphenol increased from 24 to 80% (1:16 ratio) and from 7 to 41% (1:2 ratio). These data confirmed analyte losses during sample treatment, but also noticed the need for a high soil/solvent ratio during extraction.

Although, recovery for metabolite could be considered satisfactory (80%), the effect on residue aging was checked spiking soils samples and performing the extraction after 2 h, 1 day and 4 days. No effect was observed on MCPA, but a significant decrease in recovery was obtained on aging for the metabolite, going from 104% for a freshly spiked soil sample down to 30% after 4 days. These values reveal the need for a more aggressive/efficient extraction process in the case of the metabolite (possibly due to a strong bounding to the soil components) or most probably that losses by volatility could have occurred along the time in the spiked soil. As the interest of metabolite residues in soil seems to be less important than in water due to its high volatility [12], the procedure was focused only on MCPA residues.

The final procedure was validated with two different types of soil (A and B, see Experimental section) spiked with MCPA at two levels, 0.005 and 0.05 mg/kg, performing the analysis in quintuplicate (Table 4). The procedure was found to be precise

Table 4
Validation study of the developed procedure for the determination of MCPA in soil samples (n=5)

	0.005 mg/kg		0.05 mg/kg	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Soil A	91	7	87	4
Soil B	75	10	76	7

(RSD<7%) and accurate (87–91%) for the less organogenic sample (soil A), while for soil B the procedure resulted on a small recovery decrease (75–76%) with poorer precision (RSD<10%). The slight reduction of recovery was related to a signal suppression due to the more complexity of matrix, as when we performed a 10-fold dilution of the final extract the recoveries returned to 90%.

Typical chromatograms of standard solutions and soil samples (blank and 0.005 mg/kg spiked with MCPA) are shown in Fig. 4. A limit of detection as low as 0.3 μg/kg for MCPA in soil samples was estimated from these chromatograms (soil B). As it can be seen, both soils samples selected contained ultra-traces of MCPA (soil A 2.5 μg/kg, soil B 0.3 μg/kg).

The detection of MCPA in most of ''blank'' soil and water samples used in this work at very low levels proofs the high sensitivity of LC–MS–MS. This fact was obviously related with the wide use of this herbicide in Spanish agriculture.

4. Conclusion

This work has shown that LC–ESI-MS–MS is a rapid, sensitive and selective technique for the determination of MCPA and 4-chloro-2-methylphenol in environmental samples. The method developed presents some advantages over other previously reported. Sample preparation is not necessary in water samples and it is very simple in soil samples, rendering a fast and robust method. A run

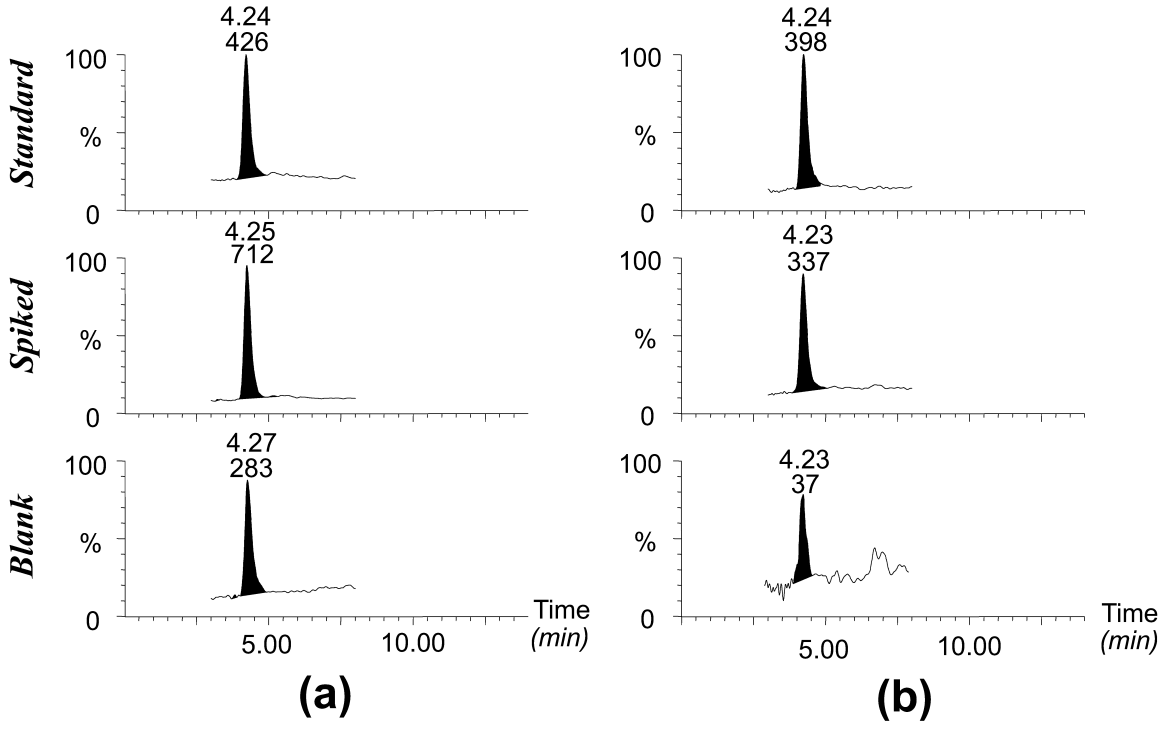

Fig. 4. LC–ESI-MS–MS chromatograms from SPE extracts of soil samples (blank and spiked at 0.005 mg/kg) and standards: (a) soil A and (b) soil B.

time of only 14 min makes this procedure rapid enough for monitoring MCPA in environmental waters at sub-μg/l levels. Good precision and recoveries were obtained, with a higher sensitivity in comparison to previously described methods. Besides, the use of (labeled) internal standard is not compulsory, as the clean-up performed by the SPE step in soil analysis is efficient enough for the removal of interferents that could suppress the analytes ionization in the electrospray source.

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