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Solid-phase microextraction liquid chromatography/tandem mass spectrometry for the analysis of chlorophenols in environmental samples

M. N. Sarrión, F. J. Santos, E. Moyano and M. T. Galceran*

Departament de Química Analítica, Facultat de Química, Universitat de Barcelona, Martí i Franquès 1-11, 08028-Barcelona, Spain

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Liquid chromatography with atmospheric pressure chemical ionisation mass spectrometry (LC/APCI-MS), using negative ion detection in a triple quadrupole instrument, was used for the determination of chlorophenols (CPs) in environmental samples. In-source collision-induced dissociation (CID) was compared with MS/MS fragmentation. In general, less fragmentation was observed in MS/MS as compared with in-source CID, with the latter providing more intense fragment ions due to chemical ionisation. Under MS/MS conditions $[M - H - HCl]^-$ was the main fragment ion observed for all compounds except for pentachlorophenol, which showed no fragmentation. For multiple reaction monitoring (MRM) acquisition mode, the transition from $[M - H]^-$ to $[M - H - HCl]^-$ was selected, leading to detection limits down to 0.3 ng injected. Direct and headspace-solid-phase microextraction (HS-SPME) were used as preconcentration procedures for the analysis of CPs in wood and in industrially contaminated soils. CPs were quantified by standard addition, which led to good reproducibility (RSD between 4 and 11%) in both SIM and MRM modes, and detection limits down to ng/g. The combination of MS/MS and in-source CID allowed confirmation of the presence of CPs in environmental samples. Copyright © 2002 John Wiley & Sons, Ltd.

Chlorophenols (CPs) can be found in environmental compartments, such as soils and waters, as compounds originating from industrial activities, or as degradation products of other pollutants like pesticides and herbicides.[1] Pentachlorophenol (PCP) has also been widely used as a preservative for wood and wood-based products. CPs are considered to be carcinogenic and are, especially in their more highly chlorinated forms, highly persistent in the environment. Some of these substances, such as 2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, and pentachlorophenol, have been included in the U.S. Environmental Protection Agency (EPA) list of 11 priority pollutant phenols in waters.[2]

Solid-phase microextraction (SPME) coupled to GC has been successfully used for the analysis of CPs from soils, directly from soil solutions,[3,4] or by headspace-SPME after *in situ* derivatisation.[5] Moreover, SPME has also been combined with accelerated solvent extraction (ASE).[6] Compared with conventional methods used for the analysis of CPs in soils and wood, SPME reduces the extraction time and solvent consumption with respect to Soxhlet extraction,[7–9] sonication[9,10] or mechanical shaking,[11] microwave-assisted extraction (MAE)[8,12] and ASE.[13] SPME is simpler than supercritical fluid extraction (SFE)[14] and can achieve detection limits in the low or sub-ng/g level.

To date, to our knowledge, SPME-LC has not yet been applied to the determination of CPs in complex matrices such as polluted soils. In a previous work,[15] the capability of an LC method with electrochemical detection for the analysis of 19 CPs in drinking water and pentachlorophenol in wood has been demonstrated. However, for very complex matrices, such as soils, the use of mass spectrometry (MS) can offer advantages over the conventional LC detectors to confirm the presence of the analytes in the sample. Liquid chromatography/mass spectrometry (LC/MS), mainly with atmospheric pressure ionisation sources (API), has been used to analyse phenols. Barceló *et al.*[16,17] and Galceran *et al.*[18] compared the performance of ESI and APCI sources for the analysis of chloro- and nitrophenolic compounds and demonstrated that APCI sources showed the best performance. Nevertheless, the application of these methods to the analysis of environmental samples such as water[19–25] and soils[7,8] makes it necessary to use extraction and preconcentration procedures.

In general, CPs show the $[M - H]^-$ ion as the base peak in the LC/MS negative ion spectra. However, for confirmation purposes, more than one ion is necessary. The fragmentation of CPs to obtain additional ions has been achieved through in-source collision-induced dissociation (CID) when a single

**Correspondence to*: M. T. Galceran, Departament de Química Analítica, Facultat de Química, Universitat de Barcelona, Martí i Franquès 1-11, 08028-Barcelona, Spain.
E-mail: galceran@apolo.qui.ub.es

quadrupole instrument is used,[7,16–18] or through tandem mass spectrometry with triple quadrupole or ion trap instruments.[26–32] Nevertheless, when in-source CID is applied, more species are produced in the fragmentation process and the spectra are more complex. Moreover, the sensitivity decreases significantly when the voltage needed to produce in-source CID increases, so limits of detection worsen. In contrast, tandem mass spectrometry allows the achievement of good detection limits due to an improvement in the signal-to-noise ratio.

At present, to our knowledge, the literature contains no reports on the simultaneous determination of all the CP isomers by LC/APCI-MS/MS with a triple quadrupole mass spectrometer. The MS/MS approach has been used only to analyse some CPs in water and soils by ESI-MS/MS[26–30] and by APCI-MS/MS[31] using a triple quadrupole instrument. Moreover, a CE/ESI-MS/MS method using an ion trap mass spectrometer[32] has been developed for the analysis of 18 CPs in water.

In this work, SPME-LC/APCI-MS/MS has been optimised for the determination of CPs in soils and pentachlorophenol in wood. First, LC/MS coupling conditions and MS/MS working parameters have been studied in order to obtain the maximum sensitivity for the analysis of these compounds using a triple quadrupole mass spectrometer. Second, the capability of direct SPME and headspace-SPME (HS-SPME) in conjunction with LC/MS were studied for soil and wood analysis. Finally, the SPME-LC/MS methods were evaluated by comparing the results with those obtained in two certification exercises.

EXPERIMENTAL

Chemicals

CPs were purchased from the following sources: 4-chlorophenol (4-CP, 99%) from Carlo Erba (Milan, Italy); 2-chlorophenol (2-CP, 98%) from Merck (Darmstadt, Germany); 3-chlorophenol (3-CP, 99%), 2,3-dichlorophenol (2,3-DCP, 98%), 2,4-dichlorophenol (2,4-DCP, 99%), 2,5-dichlorophenol (2,5-DCP, 98%), 3,4-dichlorophenol (3,4-DCP, 99%), 3,5-dichlorophenol (3,5-DCP, 99%), 2,3,4-trichlorophenol (2,3,4-TCP, 99%), 2,3,5-trichlorophenol (2,3,5-TCP, 99%), 2,3,6-trichlorophenol (2,3,6-TCP, 99%), 2,4,5-trichlorophenol (2,4,5-TCP, 99%) and 2,4,6-trichlorophenol (2,4,6-TCP, 98%) from Sigma-Aldrich (Milwaukee, WI, USA); 2,3,4,6-tetrachlorophenol (2,3,4,6-TeCP, 98%) and 2,3,5,6-tetrachlorophenol (2,3,5,6-TeCP, 96%) from Chem Service (West Chester, PA, USA); 3,4,5-trichlorophenol (3,4,5-TCP, 98.9%) and 2,3,4,5-tetrachlorophenol (2,3,4,5-TeCP, 99%) from Supelco (Bellefonte, PA); and 2,6-dichlorophenol (2,6-DCP, 99%) and pentachlorophenol (PCP, 99%) from Fluka (Buchs, Switzerland). The internal standard 3-bromophenol (3-BP, 97%) was obtained from Sigma-Aldrich.

Analytical-grade ammonium acetate ($\geq$99%) was obtained from Fluka. Methanol and acetonitrile of HPLC grade, and sodium chloride, acetic acid and hydrochloric acid of analytical grade, were supplied by Merck. Water was purified with a Elix-Milli-Q system (Millipore Corp., Beldford, MA, USA).

Individual stock standard solutions of each CP (5 mg/mL) were prepared by weight in methanol. A stock standard solution containing all the compounds at 100 μg/mL was prepared from the individual standard solutions by dilution in methanol. For optimisation of MS and MS/MS conditions and to establish quality parameters, standards were prepared by dilution with mobile phase from the 100 μg/mL stock standard solutions.

For PCP determination in the wood sample, a methanolic standard solution containing PCP at 270 μg/mL was used to spike the sample, whereas, for soil analysis, methanolic standard solutions containing all CPs at concentrations between 2 and 800 μg/mL were prepared for direct SPME and from 0.7 to 350 μg/mL for HS-SPME.

Liquid chromatography

A liquid chromatograph, Agilent 1100 series (Agilent Technologies, Palo Alto, CA, USA), equipped with an autosampler was used. An Hypersil Green C_8 ENV column 150 × 2.1 mm i.d., 3 μm particle size (Thermo Hypersil, Bellefonte, PA, USA) was used for the chromatographic separation at a flow rate of 0.2 mL/min, injecting 20 μL onto the column. A mobile phase of (A) aqueous ammonium acetate/acetic acid (5 mM, pH 4.5), (B) acetonitrile and (C) methanol, at 0.2 mL/min, was used. The elution programme started at an initial composition of 60:30:10 (v/v/v) and the isocratic step was maintained up to 29 min; then the acetonitrile content was increased to 12:86:2 in 5 min and held at this composition for 6 min. Finally, the mobile phase was returned to the initial composition in 2 min, and the column was equilibrated with the initial mobile phase composition for 10 min.

Mass spectrometry

Atmospheric pressure chemical ionisation (APCI) in negative mode was performed using a PE Sciex API 3000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, USA). The ionisation source working conditions were: vapouriser temperature, 400 °C; nebuliser current, 3 μA; declustering potential, −40 V, nebuliser and curtain gases (nitrogen), 10 and 8 arbitrary units (a.u.), respectively.

For full-scan acquisition mode, the mass spectrometer was operated over the mass range *m/z* 70–300 in centroid mode, at a cycle time of 1.2 s and an interscan time of 5 ms. To enhance sensitivity, quantitation was performed by using the selected ion monitoring (SIM) mode; the acquisition, monitoring $[M - H]^-$ ions, was time programmed in two segments from 0 to 16 min and from 16 to 45 min. In the first segment, *m/z* values corresponding to mono-CPs (MCPs), di-CPs (DCPs) and 3-BP were monitored using a dwell time of 500 ms, whereas, in the second one, ions for 3,5-DCP, tri-CPs (TCPs), tetra-CPs (TeCPs) and PCP were recorded with a dwell time of 350 ms. In both cases a SIM cycle time of 1.5 s (including an interchannel delay time of 5 ms) was used.

For multiple reaction monitoring (MRM) mode, the third quadrupole was operated at unit *m/z* resolution and the first quadrupole in low resolution. The acquisition, monitoring the transitions given in Table 1, was time programmed as in SIM mode using dwell times of 500 and 350 ms in the first and second segment, respectively, and an interchannel delay time of 5 ms. Nitrogen was used as CID gas (at 8 a.u.) and the

Table 1. Main ions (tentative assignment) and relative abundances of CPs obtained working with LC/APCI-MS in MS/MS modes under the conditions described in the Experimental Section

		MS/MS spectra				
Compound	M_W	Precursor ion (*m/z*)	Collision offset voltage (−V)	Product ion (*m/z*) (% Rel. abundance)	Assignment	MRM Transition (*m/z*)
2-CP	128	127	25	127 (100)	$[M - H]^-$	127 → 91
				91 (18)	$[M - H - HCl]^-$	
3-CP	128	127	25	127 (100)	$[M - H]^-$	127 → 91
				91 (6)	$[M - H - HCl]^-$	
4-CP	128	127	25	127 (100)	$[M - H]^-$	127 → 91
				91 (12)	$[M - H - HCl]^-$	
2,3-DCP	162	161	25	161 (100)	$[M - H]^-$	161 → 125
				125 (13)	$[M - H - HCl]^-$	
2,4-DCP	162	161	25	161 (60)	$[M - H]^-$	161 → 125
				125 (100)	$[M - H - HCl]^-$	
				89 (13)	$[M - H - 2HCl]^-$	
2,5-DCP	162	161	25	161 (100)	$[M - H]^-$	161 → 125
				125 (34)	$[M - H - HCl]^-$	
2,6-DCP	162	161	25	161 (100)	$[M - H]^-$	161 → 125
				125 (4)	$[M - H - HCl]^-$	
3,4-DCP	162	161	25	161 (94)	$[M - H]^-$	161 → 125
				125 (100)	$[M - H - HCl]^-$	
				89 (4)	$[M - H - 2HCl]^-$	
3,5-DCP	162	161	25	161 (100)	$[M - H]^-$	161 → 125
				125 (35)	$[M - H - HCl]^-$	
				89 (7)	$[M - H - 2HCl]^-$	
2,3,4-TCP	196	195	25	195 (100)	$[M - H]^-$	195 → 159
				159 (70)	$[M - H - HCl]^-$	
2,3,5-TCP	196	195	25	195 (66)	$[M - H]^-$	195 → 159
				159 (100)	$[M - H - HCl]^-$	
2,3,6-TCP	196	195	25	195 (100)	$[M - H]^-$	195 → 159
				159 (76)	$[M - H - HCl]^-$	
2,4,5-TCP	196	195	25	195 (100)	$[M - H]^-$	195 → 159
				159 (53)	$[M - H - HCl]^-$	
2,4,6-TCP	196	195	25	195 (100)	$[M - H]^-$	195 → 159
				159 (4)	$[M - H - HCl]^-$	
3,4,5-TCP	196	195	25	195 (57)	$[M - H]^-$	195 → 159
				159 (100)	$[M - H - HCl]^-$	
2,3,4,5-TeCP	230	229	30	229 (65)	$[M - H]^-$	229 → 193
				193 (100)	$[M - H - HCl]^-$	
2,3,4,6-TeCP	230	229	30	229 (100)	$[M - H]^-$	229 → 193
				193 (4)	$[M - H - HCl]^-$	
2,3,5,6-TeCP	230	229	30	229 (100)	$[M - H]^-$	229 → 193
				193 (90)	$[M - H - HCl]^-$	
PCP	264	263	30	263 (100)	$[M - H]^-$	263 → 263
3-BP (I.S.)	172	171	25	171 (5)	$[M - H]^-$	171 → 79
				79 (100)	$[Br]^-$	

collision cell offset voltage was − 25 V for all compounds, except for TeCPs for which − 30 V was used.

Wood and soil analysis by solid-phase microextraction

A wood sample containing PAHs and PCP (BCR-683) was provided by the Bundesanstalt für Materialforschung und -prüfung (BAM; Berlin, Germany), and the certified soil (CRM-530) was supplied by the Measurement and Testing Programme (M&T-BCR) of the Union of the European Communities (Brussels). CRM-530 is a clay soil contaminated by CPs, chlorobenzenes, chlorinated pesticides, aromatic carboxylic acids, chlorinated dibenzo-*p*-dioxins and dibenzofurans as a result of industrial processes. Three replicate analyses of the wood and soil sample were performed by standard addition, by adding 5–20 μL of a methanolic solution of CPs (only PCP for the wood sample). After addition of a methanolic solution of 3-BP (5 μL) used as internal standard, and of methanol up to a total volume of 25 μL if necessary, samples were equilibrated overnight at 4 °C and then extracted under the optimised conditions.

SPME experiments were performed with a manual fibre holder supplied by Supelco (Bellefonte, PA, USA), using a polar fibre (50-μm carbowax-templated resin (CW-TPR) from Supelco). Before use, the fibre was conditioned in acetonitrile with stirring for 1 h, followed by methanol (1 h). SPME experiments were performed as described in a previous work for the analysis of CPs in water and wood.[15] Briefly, Milli-Q water (20 mL) was added to the wood sample (0.75 g) in a 30-mL screw-cap glass vial. The pH was

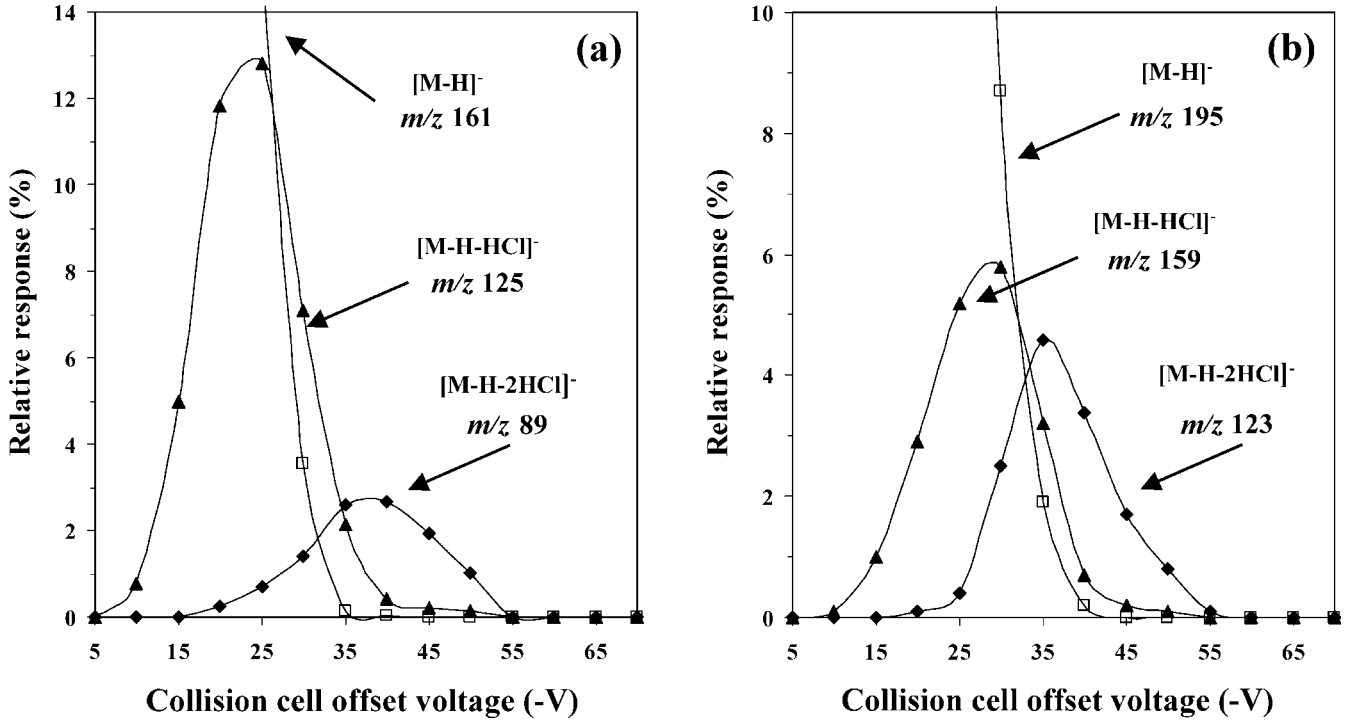

Figure 1. Effect of collision cell offset voltage on the responses obtained for product ions in MS/MS process for (a) 3,4-DCP and (b) 2,3,6-TCP.

adjusted to 3.5 with HCl (0.5 M) and the wood solution was equilibrated for 15 min at 40 °C. For soil analysis, 0.1 g of sample was acidified with 200 µL of a hydrochloric acid solution (0.5 M) for 1 h. After that, Milli-Q water (20 mL) and NaCl (6 g) were added to the vial and the soil solution was equilibrated as described for wood. After stabilisation, the soil and wood samples were extracted by immersion of the CW-TPR fibre in the sample solution for 50 min. Magnetic stirring at 1200 rpm was applied during both equilibration and extraction steps using a 10 × 5 mm Teflon-coated stir bar. The desorption of CPs was performed by immersion of the fibre in 40 µL of mobile phase (ammonium acetate/acetic acid 5 mM/acetonitrile/methanol, 60:30:10, v/v/v), placed in a conical glass insert of 0.10 mL inside a 2-mL crimp vial, for 5 min at 30 °C. 15 µL of this solution were injected in the LC/MS system. Carryover was prevented by keeping the fibre first in 4 mL of methanol (5 min) and then in 4 mL of Milli-Q water (5 min). Blanks were run periodically during the analysis to confirm the absence of contaminants.

HS-SPME analysis of soil samples was performed using a method described elsewhere for chlorobenzene with some modifications.[33] Briefly, 200 µL of a hydrochloric acid solution (0.1 M) were added to 0.04 g of soil placed in a 30-mL screw-cap glass vial, and the CW-TPR fibre was exposed to the headspace above the soil slurry at 60 °C for 50 min. The desorption process was accomplished as described previously for direct SPME.

RESULTS AND DISCUSSION

Optimisation of LC/MS conditions

To optimise the APCI conditions, different parameters affecting the ionisation efficiency and the desolvation, such as discharge needle current, vapouriser temperature and nebuliser done on disc gas and curtain gas flow rates, were examined by flow injection of some CPs (1 µg/mL). The optimal conditions are given in the Experimental section.

The declustering potential, which affects the desolvation and fragmentation processes in the API interface, was optimised between − 200 and 0 V using infusion of individual solutions of each CP at 20 µg/mL prepared in mobile phase. The optimum potential value is compound-dependent. At low potentials, clusters formed with components from the mobile phase are favoured and the abundance of the $[M - H]^-$ ion decreases. At high declustering potentials in-source CID can occur and the abundance of the $[M - H]^-$ ion is reduced. As a compromise between maximum response and low degree of fragmentation to obtain appropriate sensitivity in SIM mode, a value of − 40 V was chosen for all the compounds; a low level of fragmentation was thus observed, and the spectra were always dominated by the isotopic pattern of the $[M - H]^-$ ions. At this potential the isotopic pattern of ions corresponding to the loss of HCl was also observed with relative abundances (R.A.) of <5–22% for DCPs, between 13 and 27% for TCPs and slightly higher for TeCPs (25–61%). PCP showed two important isotopic ion patterns, corresponding to the $[M - H]^-$ ions (*m/z* 263, 70%), and to $[M - Cl]^-$ ions (*m/z* 229, 29%). Moreover, additional losses of HCl for TeCPs and PCP were observed, although at low relative abundances (<5 and 11%). These results generally agree with those previously published.[16,18]

For quantitation purposes the $[M - H]^-$ ion was chosen for SIM acquisition mode as well as precursor ion for

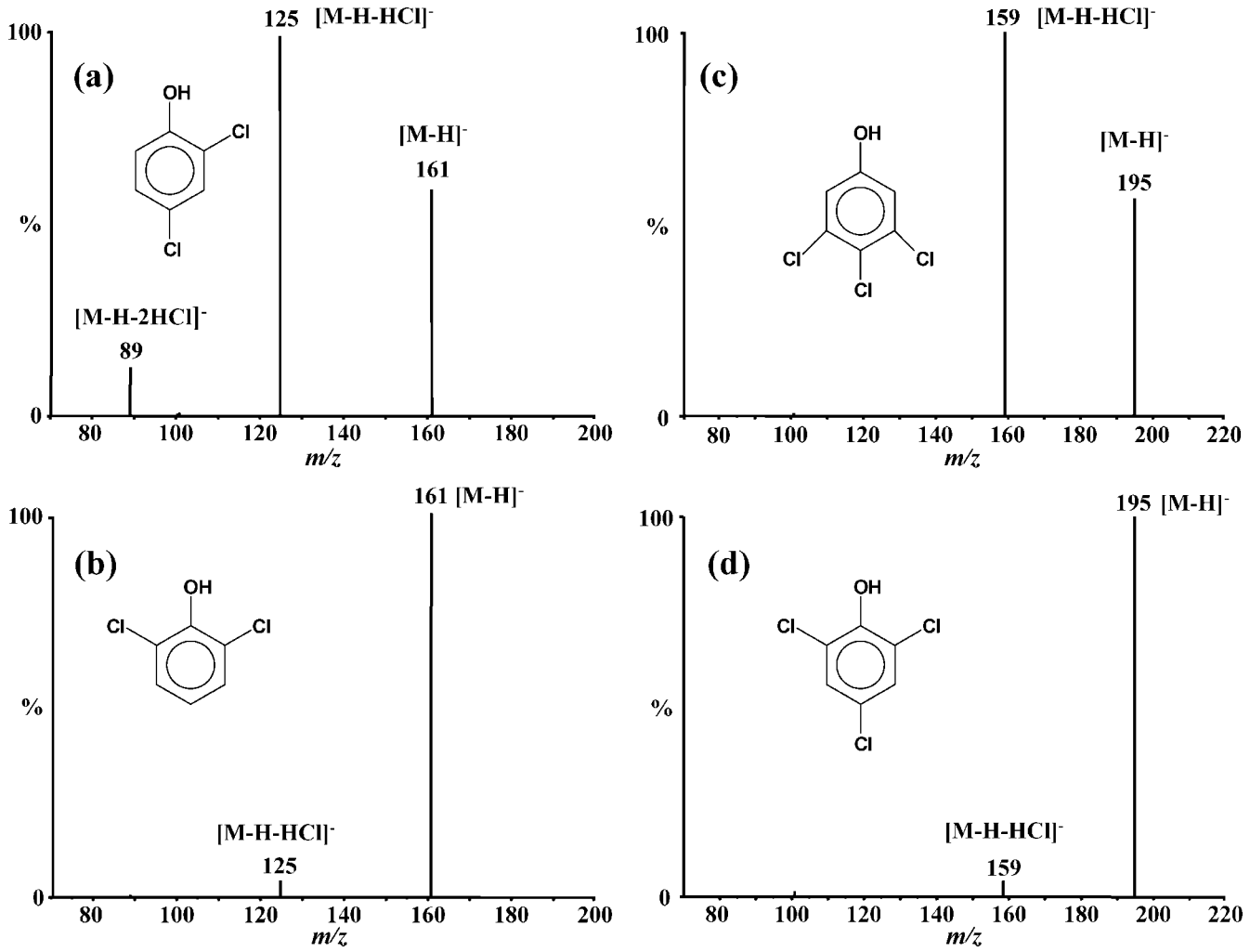

Figure 2. LC/MS/MS product ion spectra corresponding to the isomer pairs: (a) 2,4-DCP and (b) 2,6-DCP; and (c) 3,4,5-TCP and (d) 2,4,6-TCP. The collision cell offset voltage was −25 V.

MS/MS studies (Table 1). The effects of the CID gas pressure and the collision cell voltage were studied by infusion of each CP in turn, in order to optimise the working MS/MS conditions. The CID gas pressure was varied from 4 to 12 a.u. and the optimum value was chosen as a compromise between the maximum transmission of the precursor ion and the pressure needed to produce fragmentation. For CPs, CID gas pressures between 8 and 10 a.u. provided the best responses. Since a decrease in the response for PCP (15%) was observed at 10 a.u., 8 a.u. was used for further studies.

The product ion spectra obtained depend on the collision cell offset voltage, which was varied from 0 to − 180 V. In general, the loss of HCl for CPs was the most intense fragment ion observed at low offset voltages (absolute value, i.e. low collision energy), whereas at high absolute values the loss of a second molecule of HCl due to multicollisional fragmentation occurred for DCPs and TCPs, giving fragment ions with low abundance (Fig. 1). The $[M - H - 2HCl]^-$ ion has also been observed in an ion trap instrument when acquiring the MS^3 spectra for DCPs and TCPs.[32] In our triple quadrupole, TeCPs did not show any additional fragmentation at high absolute collision cell voltages. It must be mentioned that, in general, fragmentation of CPs under MS/MS conditions was poor. Only for five of the nineteen CPs (Table 1) was the base peak the fragment ion $[M - H - HCl]^-$. Moreover, the optimum absolute value of offset voltage increased with degree of chlorination. For instance, compounds containing up to three halogen atoms required lower collision energies than TeCPs, and PCP was not fragmented even at high values. Under identical collision cell voltages, MS/MS spectra of isomers differ significantly in the relative abundances of their product ions, so MS/MS can help to distinguish CP isomers. As an example, the product ion spectra obtained at − 25 V for 2,4-DCP and 2,6-DCP and for 3,4,5-TCP and 2,4,6-TCP are shown in Fig. 2.

The fragmentation observed in the MS/MS experiments for the CPs (Table 1) generally agrees with that previously published. For instance, Marchese *et al.*[28] and Bossi *et al.*[30] reported poor fragmentation for 2,4-DCP and a high activation amplitude was necessary to fragment 2,4,6-TCP using an ion trap.[32] Finally, the non-fragmentation of PCP has also been reported by Kienhuis *et al.*[31] and Jáuregui *et al.*[32]

As mentioned above, in-source CID can be used to obtain some structural information using quadrupole instruments. Comparing in-source CID fragmentation spectra with those

Table 2. Quality parameters of LC-MS

		SIM				MRM			
				Precision (%)[b]				Precision (%)[b]	
Compound	Peak No.	Linear range[a] (ng inj.)	LOD (pg inj.)	Run-to-run[c]	Day-to-day[d]	Linear range[e] (ng inj.)	LOD (pg inj.)	Run-to-run[c]	Day-to-day[d]
2-CP	1	0.20–20	54	1.3	2.9	0.05–130	13	2.3	4.0
3-CP	3	0.10–20	32	2.1	2.5	0.05–100	12	3.3	3.5
4-CP	2	0.10–15	33	2.1	3.7	0.04–100	4	2.0	2.4
2,3-DCP	5	0.07–40	11	2.0	2.6	0.03–125	7	1.5	3.3
2,4-DCP	7	0.07–40	11	1.7	3.9	0.02–100	4	1.2	2.0
2,5-DCP	6	0.06–40	12	1.3	2.9	0.01–100	3	3.0	3.3
2,6-DCP	4	0.06–40	9	1.7	1.9	0.06–150	14	2.5	4.4
3,4-DCP	8	0.04–40	7	2.1	3.0	0.007–90	2	1.0	1.3
3,5-DCP	10	0.04–40	15	2.1	2.5	0.01–100	2	1.4	2.2
2,3,4-TCP	12	0.07–40	18	2.2	3.4	0.02–180	4	1.5	2.5
2,3,5-TCP	14	0.06–50	20	2.0	3.3	0.02–180	5	1.0	2.3
2,3,6-TCP	9	0.06–40	15	1.7	2.2	0.03–150	5	1.3	3.5
2,4,5-TCP	13	0.06–40	18	2.3	2.7	0.01–150	3	2.1	3.6
2,4,6-TCP	11	0.06–50	15	2.3	2.8	0.25–250	62	2.2	4.9
3,4,5-TCP	16	0.06–40	15	1.5	2.8	0.01–150	2	1.5	3.3
2,3,4,5-TeCP	19	0.07–15	5	3.0	5.1	0.005–70	0.3	1.3	3.3
2,3,4,6-TeCP	17	0.15–50	45	2.8	3.9	0.60–350	175	5.7	6.6
2,3,5,6-TeCP	15	0.15–50	43	3.0	3.7	0.02–280	3	1.7	3.0
PCP	18	0.06–50	9	2.8	5.2	0.03–150	8[f]	1.9	2.7

[a] Correlation coefficients (r^2), 0.997–0.999;
[b] Concentration level: 100 μg/L;
[c] n = 5;
[d] n = 3 replicates ×3 days;
[e] Correlation coefficients (r^2), 0.998–0.999;
[f] m/z 263 → m/z 263.

obtained by MS/MS, a higher level of fragmentation was observed by in-source CID. The most important differences were observed for 2,3,4,6-TeCP and PCP. The TeCP showed good fragmentation by in-source CID whereas very poor fragmentation occurred in MS/MS (Table 1). Moreover, as has been mentioned, while PCP did not show fragmentation in MS/MS, by in-source CID both $[M - Cl]^-$ and $[M - Cl - HCl]^-$ ions were observed. It must be taken into account that in-source CID is dependent on mobile phase composition, and the fragmentation behaviour observed for PCP and 2,3,4,6-TeCP can be rationalised by collisional activation mechanisms involving some mobile phase components.

In spite of the poor degree of fragmentation obtained in MS/MS, in-source CID can be combined with MS/MS to obtain additional information that would help in the identification of CPs in complex matrixes. For instance, two transitions can be monitored for TeCP, one using $[M - H]^-$ as precursor ion, $[M - H]^- \rightarrow [M - H - HCl]^-$ (m/z 229 → 193), and the other using a fragment obtained in the source, $[M - H - HCl]^- \rightarrow [M - H - 2HCl]^-$ (m/z 193 → 157). However, for TeCPs, the transition (m/z 195 → 159) corresponding to one ^{37}Cl atom in the ion was selected due to the coincidence of these m/z values with those selected for TCPs, thus allowing a reduction in the number of transitions monitored for the analysis.

Finally, to achieve good sensitivity in MRM acquisition mode, the collision cell voltage was chosen to maximise the intensity of the $[M - H - HCl]^-$ ions. As a compromise, an offset voltage of − 25 V was chosen for MCPs, DCPs and

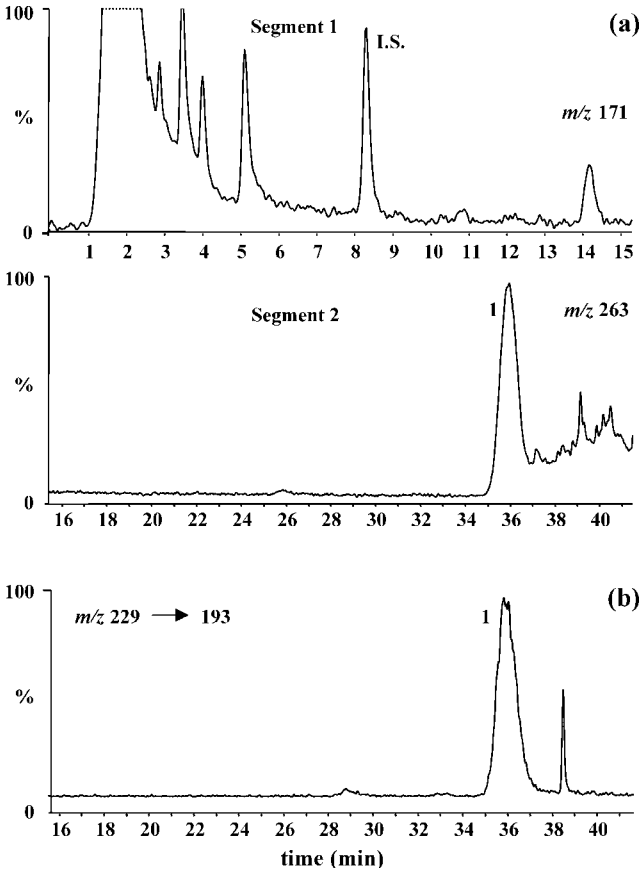

Figure 3. SPME-LC/APCI-MS chromatograms corresponding to the wood sample in (a) SIM and (b) MRM mode. Peaks: 1 = PCP and I.S. = 3-BP.

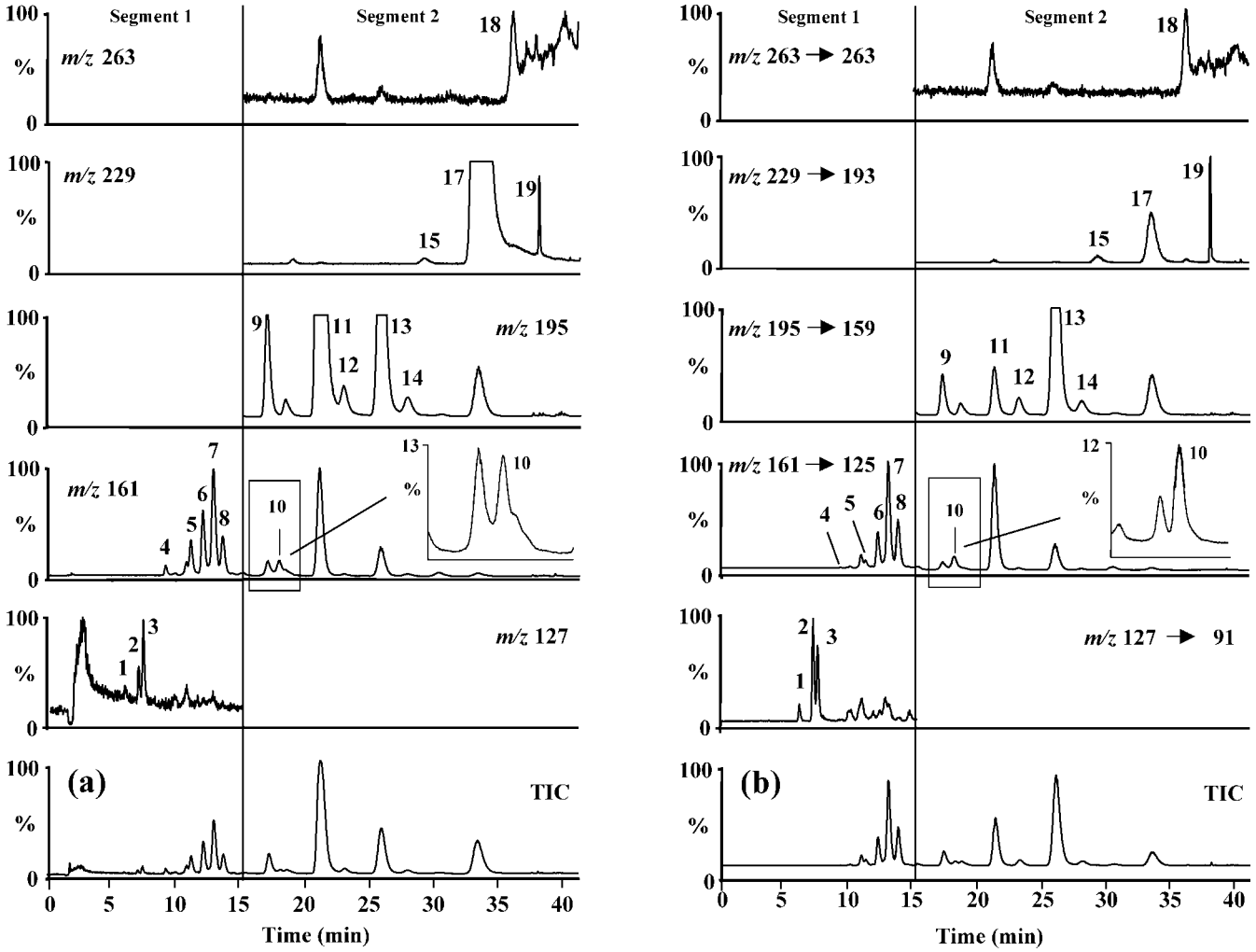

Figure 4. Direct SPME-LC/APCI-MS chromatogram of the CRM-530 soil in (a) SIM and (b) MRM mode. Peaks: 1: 2-CP; 2: 4-CP; 3: 3-CP; 4: 2,6-DCP; 5: 2,3-DCP; 6: 2,5-DCP; 7: 2,4-DCP; 8: 3,4-DCP; 9: 2,3,6-TCP; 10: 3,5-DCP; 11: 2,4,6-TCP; 12: 2,3,4-TCP; 13: 2,4,5-TCP; 14: 2,3,5-TCP; 15: 2,3,5,6-TeCP; 17: 2,3,4,6-TeCP; 18: PCP; 19: 2,3,4,5-TeCP.

TCPs, and a value of −30 V for TeCPs. Relative abundances of product ions for each compound at the optimised conditions, and the transitions used for quantification in MRM, are given in Table 1.

Quality parameters

Quality parameters for LC/MS and LC/MS/MS methods in SIM and MRM modes, respectively, were determined (Table 2). The linearity was examined for standard solutions (between 5 pg and 1 μg injected), using 3-BP as internal standard. In SIM mode, the response for the standards was found to be linear over a shorter concentration range (from 0.04–50 ng injected) compared with MRM (from 0.01–350 ng injected), but in both cases correlation coefficients (r^2) were higher than 0.997 for all compounds. Run-to-run precision (n = 5) of the LC/MS method was studied using a standard solution of 0.2 μg/mL for each phenol. In addition, three injections on three different days were performed to establish day-to-day precision. Run-to-run precision (RSD between 1.0–5.7%) and day-to-day precision (RSD from 1.3–6.6%) were similar for SIM and MRM modes (Table 2). In general, LODs (S/N = 3) in MRM mode (0.3–14 pg) were lower than those obtained in SIM mode (5–54 pg) except for 2,4,6-TCP and 2,3,4,6-TeCP, which showed low fragmentation efficiency in MS/MS experiments (Table 2).

Analysis of wood by direct SPME

To examine the feasibility of the LC/MS method, PCP was analysed in a certified wood sample (BCR-683) by direct SPME. Standard addition was used to avoid matrix effects and 3-BP was chosen as internal standard. The ion chromatogram for the wood sample in SIM mode using $[M - H]^-$ (*m/z* 263) as diagnostic ion is given in Fig. 3(a). Moreover, in-source CID-MS/MS has also been performed to confirm the presence of PCP. The chromatogram corresponding to the transition from the ion $[M - Cl]^-$ (*m/z* 229), formed in the source, to the ion $[M - Cl - HCl]^-$ (*m/z* 193) obtained by MS/MS is shown in Fig. 3(b). SPME-LC/MS is a highly selective procedure for the analysis of PCP in wood, because no interferences from other compounds potentially present in the sample matrix were observed. The mean value obtained with direct SPME in conjunction with LC/MS in SIM mode (3.57 ± 0.20 μg/g; n = 3) was in

Table 3. Detection limits and precision of the proposed SPME-LC-MS method in the analysis of soil

	LOD[a] (ng/g)				Run-to-run precision (R.S.D. %) ($n = 5$)	
	Direct-SPME		HS-SPME		Direct-SPME	HS-SPME
Compound	SIM	MRM	SIM	MRM	SIM (MRM)	SIM (MRM)
2-CP	920	25	217	9	–[b] (5)	8 (9)
3-CP	440	25	132	7	6 (5)	4 (5)
4-CP	454	8	115	2	6 (5)	5 (6)
2,3-DCP	11	12	5	6	7 (6)	4 (12)
2,4-DCP	6	1	3	0.5	8 (5)	4 (7)
2,5-DCP	11	4	6	2	8 (7)	9 (10)
2,6-DCP	20	28	15	14	9 (7)	6 (8)
3,4-DCP	13	1	11	1	7 (6)	4 (10)
3,5-DCP	12	4	8	3	–[c] (6)	–[c] (4)
2,3,4-TCP	20	10	7	3	9 (8)	6 (5)
2,3,5-TCP	51	10	14	3	9 (7)	6 (5)
2,3,6-TCP	17	7	8	3	6 (5)	10 (5)
2,4,5-TCP	14	3	8	1	8 (9)	5 (6)
2,4,6-TCP	1	6	1	2	8 (8)	11 (11)
3,4,5-TCP	53	5	50	6	n.d.	n.d.
2,3,4,5-TeCP	40	1	13	0.4	10 (11)	6 (5)
2,3,4,6-TeCP	5	20	2	8	10 (10)	11 (4)
2,3,5,6-TeCP	47	6	15	2	9 (11)	5 (11)
PCP	103	–	18	–	12 (12)	8 (8)

[a] LOD = Limit of detection calculated with 0.1 g of agricultural soil.
[b] compound height at LOD level.
[c] interferred peak.
n.d. = not detected.

good agreement with those obtained by six European laboratories that participated in the certification exercise (3.64 ± 0.19 μg/g, $n = 6$). Run-to-run precision (RSD) of the SPME-LC/MS method for PCP in the wood sample was 5% (n = 5), whereas LOD (S/N = 3, SIM mode), experimentally estimated from wood samples free of detectable quantities of PCP spiked at low concentration levels (below LOQ), was 15 ng/g.

Table 4. Analysis of CPs in CRM-530 soil by SPME-LC-APCI-MS

	Concentration (μg/g)						
	Direct-SPME (n = 3)		HS-SPME (n = 3)		Significance level (P-value)[a]		Certified value
	SIM (A)	MRM (B)	SIM (C)	MRM (D)			
Compound	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	A vs. C	B vs. D	Mean ± S.D.
2-CP	<LOQ	1.5 ± 0.1	1.4 ± 0.1	1.7 ± 0.2	–	0.096	–
3-CP	6.8 ± 0.4	6.6 ± 0.8	6.4 ± 0.3	6.5 ± 0.3	0.313	0.606	6.7 ± 0.8
4-CP	2.9 ± 0.1	3.1 ± 0.2	2.8 ± 0.2	2.8 ± 0.2	0.615	0.165	–
2,3-DCP	6.6 ± 0.2	6.3 ± 0.1	6.4 ± 0.2	6.4 ± 0.5	0.312	0.757	–
2,4-DCP	31 ± 3	32 ± 3	32 ± 1	33 ± 3	0.693	0.750	–
2,5-DCP	17 ± 1	16 ± 2	20 ± 2	16 ± 2	0.081	0.695	–
2,6-DCP	2.4 ± 0.2	2.5 ± 0.2	2.4 ± 0.2	2.2 ± 0.2	0.936	0.167	–
3,4-DCP	5.9 ± 0.2	5.8 ± 0.4	6.4 ± 0.3	6.1 ± 0.7	0.059	0.572	6.0 ± 0.5
3,5-DCP	–[b]	1.4 ± 0.1	–[b]	1.5 ± 0.1	–	0.134	–
2,3,4-TCP	2.6 ± 0.2	2.4 ± 0.2	2.7 ± 0.2	2.6 ± 0.1	0.716	0.233	–
2,3,5-TCP	2.6 ± 0.2	2.5 ± 0.3	2.7 ± 0.1	2.6 ± 0.1	0.437	0.258	–
2,3,6-TCP	11 ± 1	11 ± 1	11 ± 1	11 ± 1	0.874	0.632	–
2,4,5-TCP	38 ± 3	41 ± 3	44 ± 5	44 ± 3	0.138	0.313	40 ± 7
2,4,6-TCP	204 ± 5	198 ± 16	201 ± 26	212 ± 27	0.842	0.492	–
3,4,5-TCP	n.d.	n.d.	n.d.	n.d.	–	–	–
2,3,4,5-TeCP	0.43 ± 0.03	0.42 ± 0.04	0.41 ± 0.03	0.46 ± 0.03	0.435	0.322	–
2,3,4,6-TeCP	81 ± 4	82 ± 4	81 ± 10	80 ± 2		0.476	76 ± 9
2,3,5,6-TeCP	0.17 ± 0.01	0.15 ± 0.01	0.17 ± 0.01	0.16 ± 0.02	0.825	0.548	–
PCP	0.54 ± 0.03	0.50 ± 0.04	0.54 ± 0.06	0.51 ± 0.04	0.995	0.765	0.47 ± 0.08

[a] Significant differences between procedures for $P < 0.05$ (at the 95% confidence level).
[b] peak interferred at m/z 161, n.d. = not detected.
LOQ = limit of quantification.

Analysis of soil by direct SPME and headspace-SPME

The analysis of CPs present in the soil CRM-530 was performed by both direct and HS-SPME using LC/MS and LC/MS/MS. For direct SPME, an extraction temperature of 40 °C and a time of 50 min were used. For HS-SPME, the extraction temperature was increased up to 60 °C to enhance the mass transfer process, to increase the vapour pressure of CPs in the headspace and to favour the kinetics of the process. For all CPs, HS-SPME at 60 °C led to responses 1–5-fold higher than direct SPME. As an example, the chromatograms obtained by direct SPME in SIM and MRM modes are shown in Fig. 4. MRM and in-source CID-MS/MS allowed the confirmation of the CPs present in the sample. For MCPs, a better signal-to-noise ratio was achieved in the MRM ion chromatogram, allowing the determination of 2-CP that was not possible in SIM mode. Moreover, an interference in the tail of 3,5-DCP (peak number 10) at *m/z* 161 was observed in SIM mode using both SPME procedures. This interference can be avoided using MRM mode (Fig. 4(b)).

LODs in soils (S/N = 3) were estimated using an agricultural clay soil free of CPs and spiked at low μg/g level. In SIM mode, they ranged from 1 ng/g to 0.9 μg/g for direct SPME (Table 3), and the worst LOD values were obtained for MCPs, probably due to the lower affinity of these compounds for the fibre. The high value for PCP can be explained by its high K_{ow} (5.01) and low solubility in water (18 mg/L), which favoured its adsorption to the organic matter of the soil. LOD values in MRM mode were lower than in SIM mode, except for those compounds with low degrees of fragmentation, i.e., 2,6-DCP, 2,4,6-TCP and 2,3,4,6-TeCP (Table 3). The use of HS-SPME achieved lower LODs, for instance, down to 0.4–14 ng/g in MRM mode. Run-to-run precision (n = 5) of the SPME-LC/MS method gave RSD values below 12% in both detection modes, SIM and MRM, and for the two SPME methods, direct and HS-SPME (Table 3).

The results obtained in the SPME analysis of the CRM-530 soil with direct SPME and HS-SPME are given in Table 4, where the mean and standard deviation (SD) values of CPs certified in the soil are also given. The results obtained are in accordance with the certified values. The analytical significance of the mean values of both SPME strategies (direct or headspace) was statistically studied using Student's t-test. When unequal variances (*F*-test) were obtained, Cochran's test was applied. The significance values (*P*) obtained comparing the two procedures are given in Table 4. For all CPs no significant differences were observed between direct and HS-SPME methods ($P > 0.05$). Nevertheless, headspace-SPME showed some advantages over direct SPME, such as the longer durability of the fibres because of the avoidance of direct contact between fibre and polluted soil, and better LOD values for most of the compounds.

CONCLUSIONS

An LC/APCI-MS method was developed for the determination of CPs in both SIM and MRM modes. The LC/MS spectra were dominated by the $[M - H]^-$ ion and, in general, the CPs showed poor fragmentation in MS/MS. The isomers showed significant differences in the relative abundances of fragment ions under identical collision cell voltage. These ions can be used to assist with compound identification. Moreover, in-source CID fragmentation was high enough to be combined with MS/MS for confirmation purposes. Both SIM and MRM modes gave good linearity and reproducibility (run-to-run precision between 1.3 and 5.7% and day-to-day precision between 1.0 and 6.6%), achieving good detection limits. For 2,6-DCP, 2,4,6-TCP, 2,3,4,6-TeCP and PCP, all of which showed poor fragmentation in MS/MS, the SIM mode is recommended, while, for the other CPs, the MRM mode is proposed. The method has been evaluated for the analysis of wood and soils by SPME and the results show that this preconcentration technique in conjunction with LC/APCI-MS can be proposed for the analysis of CPs in environmental samples.

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REFERENCES

1. Jensen J. *Rev. Environ. Contam. Toxicol.* 1996; **146**: 25.
2. *Sampling and Analysis Procedure for Screening of Industrial Effluents for Priority Pollutants*, US Environmental Protection Agency, Environment Monitoring and Support Laboratory: Cincinnati, OH, 1977.
3. Lee M-R, Yeh Y-C, Hsiang W-S, Hwang B-H. *J. Chromatogr. A* 1998; **806**: 317.
4. Baciocchi R, Attinà M, Lombardi G, Boni MR. *J. Chromatogr. A* 2001; **911**: 135.
5. Llompart M, Blanco B, Cela R. *J. Microcol. Sep.* 2000; **12**: 25.
6. Wennrich L, Popp P, Möder M. *Anal. Chem.* 2000; **72**: 546.
7. Jáuregui O, Moyano E, Galceran MT. *J. Chromatogr. A* 1998; **823**: 241.
8. Alonso MC, Puig D, Silgoner I, Grasserbauer M, Barceló D. *J. Chromatogr. A* 1998; **823**: 231.
9. Buhr A, Genning C, Salthammer T. *Fresenius' J. Anal. Chem.* 2000; **367**: 73.
10. Lopez-Avila V, Young R, Teplitsky N. *J. Assoc. Off. Anal. Chem.* 1996; **79**: 142.
11. Frébortová J. *Biosci. Biotechnol. Biochem.* 1995; **59**: 1930.
12. Crespín MA, Gallego M, Valcárcel M. *J. Chromatogr. A* 2000; **897**: 279.
13. Pörschmann J, Plugge J, Toth R. *J. Chromatogr. A* 2001; **909**: 95.
14. Santos FJ, Jáuregui O, Pinto FJ, Galceran MT. *J. Chromatogr. A* 1998; **823**: 249.
15. Sarrión MN, Santos FJ, Galceran MT. *J. Chromatogr. A* 2002; **947**: 155.
16. Puig D, Barceló D, Silgoner I, Grasserbauer M. *J. Mass Spectrom.* 1996; **31**: 1297.
17. Puig D, Silgoner I, Grasserbauer M, Barceló D. *Anal. Chem.* 1997; **69**: 2756.
18. Jáuregui O, Moyano E, Galceran MT. *J. Chromatogr. A* 1997; **787**: 79.
19. Castillo M, Puig D, Barceló D. *J. Chromatogr. A* 1997; **778**: 301.
20. Castillo M, Barceló D. *Anal. Chem.* 1999; **71**: 3769.
21. Farré M, Pasini O, Alonso MC, Castillo M, Barceló D. *Anal. Chim. Acta* 2001; **426**: 155.
22. Azevedo DA, Lacorte S, Vinhas T, Viana P, Barceló D. *J. Chromatogr. A* 2000; **879**: 13.
23. Azevedo DA, Lacorte S, Vinhas T, Viana P, Barceló D. *Chromatographia* 2001; **53**: 113.
24. Wissiack R, Rosenberg E, Grasserbauer M. *J. Chromatogr. A* 2000; **896**: 159.
25. Wissiack R, Rosenberg E. *J. Chromatogr. A* 2002; **963**: 149.

26. Hughes BM, McKenzie DE, Duffin KL. *J. Am. Soc. Mass Spectrom.* 1993; **4**: 604.
27. Hernández F, Hidalgo C, Grimalt S, Sancho JV. *Quim. Anal.* 2001; **20**: 81.
28. Marchese S, Perret D, Gentili A, D'Ascenzo G, Faberi A. *Rapid Commun. Mass Spectrom.* 2002; **16**: 134.
29. Køppen B, Spliid NH. *J. Chromatogr. A* 1998; **803**: 157.
30. Bossi R, Vejrup KV, Mogensen BB, Asman WAH. *J. Chromatogr. A* 2002; **957**: 27.
31. Kienhuis PGM, Geerdink RB. *Trends Anal. Chem.* 2000; **19**: 460.
32. Jáuregui O, Moyano E, Galceran MT. *J. Chromatogr. A* 2000; **896**: 125.
33. Sarrión MN, Santos FJ, Galceran MT. *J. Chromatogr. A* 1998; **819**: 197.