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Capillary electrophoresis–electrospray ion-trap mass spectrometry for the separation of chlorophenols

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Abstract

Eighteen positional isomers of chlorophenols were separated by capillary electrophoresis (CE) and detected on-line by electrospray ionization ion-trap mass spectrometry (MS). Conditions for the coupling of CE to MS, e.g., the concentration of carrier electrolyte, the sheath liquid composition and the sheath gas flow-rate were optimized. Diethylmalonic acid (5 m*M*) at pH 7.25 and isopropanol–250 m*M* dimethylamine (80:20) as sheath liquid were used. The activation parameters for ion-trap mass spectrometric analysis of chlorophenols were optimized. The mass spectra, obtained for all the analytes, revealed that the $[M-H]^-$ ion was the base peak for all chlorophenols. Moreover, conditions for CE–MS–MS detection were established and $[M-H-HCl]^-$ ions were detected.

Keywords: Chlorophenols; Phenols

1. Introduction

Chlorophenols are pollutants expelled into waters and soils by numerous industrial processes [1,2]. For the analysis of phenolic compounds in environmental samples, both gas chromatography (GC) [3,4] and liquid chromatography (LC) [5–7] have been extensively used. However, the development of capillary electrophoresis (CE) has provided high resolutions, highly efficient separations, rapid analyses and low chemical and solvent consumption. Because of their acidity, phenols can be analyzed as anions under CE conditions and also as neutrals under micellar electrokinetic chromatography (MEKC) conditions. The 11 priority pollutant phenols listed by the US Environmental Protection Agency (EPA) [8] were separated by CE in fused-silica capillaries, mainly with phosphate and/or borate buffers and with UV, electrochemical or fluorescence detection [9–13]. MEKC was also applied to EPA phenols, using sodium dodecyl sulfate (SDS) as surfactant and phosphate–borate as carrier electrolyte [14]. The separation of the positional isomers of chlorophenols has been well-resolved by MEKC [15,16] and is possible when a phosphate–borate buffer with SDS as surfactant is used. Co-electroosmotic CE has also been used to separate various chlorophenols by Liu and Frank [17] using different hexadimetrines (1,5-dimethyl-1,5-diazaundecamethylene polymethobromide) to reverse the electroosmotic flow direction. Nevertheless, the separation of all the positional isomers by counterelectroosmotic CE is an unresolved question that has been studied by several researchers. For instance, CE and UV detection were used by Gonnord and Collet [10], who separated 13 chlorinated phenols and by Jáuregui et al. [18], who separated 16. However, the on-line combination of high-efficiency separations in CE and the detection

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and identification potential of MS combines the advantages of both techniques and provides a valuable tool for the separation and identification of phenols. Although the flow-rate of CE buffer is very low, the presence of non-volatile buffer constituents can hinder the (long-term) performance of MS detection, owing to interface and ion source contamination. Tsai and Her have reported a CE–MS separation for the 11 EPA priority pollutant phenols using 2-(*N*-cyclohexylamino)ethanesulfonic acid (CHES) as running buffer and water–2-propanol (20:80) containing 0.54% ammonia as sheath liquid [19]. Several volatile carrier electrolytes have been evaluated for the separation of 17 chlorophenols and the effects of electrolyte concentration and pH on the electrophoretic mobilities have been studied [18]. In the present work, we optimized the conditions for the coupling of CE with MS using an ion-trap analyzer for the separation of 18 positional isomers of chlorophenols. Parameters such as carrier electrolyte concentration and sheath gas flow-rate, sheath liquid composition and flow-rate, were studied. MS–MS parameters (activation amplitude, activation *Q* and activation time) were also studied.

2. Experimental

2.1. Chemicals

The reagents used for the preparation of buffers were of analytical grade. Diethylmalonic acid (DEM) was from Aldrich (Milwaukee, WI, USA). The buffer solutions were filtered through a 0.45-μm nylon filter and degassed before use. Ammonia (25%) and sodium hydroxide were from Merck (Darmstadt, Germany). Water was purified using a Milli-Q Elix system (Millipore, Milford, MA, USA). HPLC-grade methanol was from Merck. The phenolic compounds studied were obtained from the following sources: 2-chlorophenol, 3-chlorophenol, 2,3-dichlorophenol, 2,4-dichlorophenol, 2,5-dichlorophenol, 2,6-dichlorophenol, 3,4-dichlorophenol, 3,5-dichlorophenol, 2,3,4-trichlorophenol, 2,3,5-trichlorophenol, 2,3,6-trichlorophenol, 2,4,5-trichlorophenol and 2,4,6-trichlorophenol, all from Aldrich; 4-chlorophenol from Carlo Erba (Milan, Italy); 2,3,4,6-tetrachlorophenol, 2,3,5,6-tetrachlorophenol and pentachlorophenol from Chem Service (West Chester, PA, USA), 3,4,5-trichlorophenol from Supelco (Bellefonte, PA, USA). 2,3,4,5-Tetrachlorophenol has not been included in this study since it is not available. The abbreviations and the pK_a values for the phenolic compounds are given in Table 1. Stock solutions (500 μg ml^{-1}) of individual standards were prepared in methanol. A mixed stock solution (10 μg ml^{-1} of each compound) containing all the standards was prepared from individual standards by diluting with water until a water–methanol ratio of 4:1 was reached.

2.2. CE system

The experiments were performed on a P/ACE System 5500 (Beckman Instruments, Fullerton, CA, USA). A fused-silica capillary (Supelco), 80 cm×75 μm I.D.×360 μm O.D. was used. The temperature was kept at 25°C and the potential applied at 20 kV. Hydrodynamic injection mode (by vacuum, 20 p.s.i.; 1 p.s.i.=6894.76 Pa) was applied for 4 s. The electrolyte working solution was 5 m*M* diethylmalonic acid at pH 7.25 and was prepared daily. The pH was adjusted to the desired value with 5% NH_3. A new fused-silica capillary was pre-treated with 1 *M* NaOH for 15 min and then rinsed with ultrapure water for 15 min. The capillary was conditioned with the running electrolyte for 30 min before the first run and for 1 min between runs. Before each session, the capillary was treated with 0.1 *M* NaOH for 10 min, ultrapure water for 2 min and it was finally conditioned with the running electrolyte for 30 min.

2.3. MS system

MS was performed using an LCQ (Finnigan MAT, San José, CA, USA) ion-trap mass spectrometer equipped with a tricoaxial pneumatically assisted electrospray (ESI) ion source. The working conditions for ESP were: temperature for the heated capillary, 175°C, electrospray capillary potential, −3.5 kV and electrospray current, 12 μA. The sheath liquid was isopropanol–250 m*M* dimethylamine (80:20) at a flow-rate of 5 μl min^{-1}. The sheath gas (nitrogen) flow-rate was 13.5 l h^{-1}. Full-scan data acquisition was performed scanning from m/z 100 to 300 in centroid mode using a maximum injection

Table 1
Chlorinated phenolic compounds and diagnostic ions in MS infusion studies and product ions in MS–MS and MS^3

Compound	Abbreviation	Peak No.	pK_a	M_r	MS, diagnostic ion $[M-H]^-$	MS–MS, product ion $[M-H-HCl]^-$	MS^3, product ion $[M-H-2HCl]^-$
2-Chlorophenol	2CP	1	8.1	128	127	91	ND
3-Chlorophenol	3CP	2	8.9	128	127	91	ND
4-Chlorophenol	4CP	3	9.4	128	127	91	ND
2,3-Dichlorophenol	23DCP	4	7.7	162	163	127	91
2,4-Dichlorophenol	24DCP	5	7.7	162	163	127	91
2,5-Dichlorophenol	25DCP	6	7.5	162	163	127	91
2,6-Dichlorophenol	26DCP	7	6.8	162	163	127	91
3,4-Dichlorophenol	34DCP	8	8.6	162	163	127	91
3,5-Dichlorophenol	35DCP	9	8.2	162	163	127	91
2,3,4-Trichlorophenol	234TCP	10	6.9	196	195	159	123
2,3,5-Trichlorophenol	235TCP	11	6.4	196	195	159	123
2,3,6-Trichlorophenol	236TCP	12	5.8	196	195	159	123
2,4,5-Trichlorophenol	245TCP	13	6.7	196	195	159	123
2,4,6-Trichlorophenol	246TCP	14	6.7	196	195	159	123
3,4,5-Trichlorophenol	345TCP	15	7.5	196	195	159	123
2,3,4,6-Tetrachlorophenol	2346TeCP	16	5.2	230	229	193	123
2,3,5,6-Tetrachlorophenol	2356TeCP	17	5.0	230	229	193	123
Pentachlorophenol	PCP	18	4.9	264	263	ND	ND

ND: Not detected.

time of 200 ms and performing 1 μscan. MS–MS was performed with an isolation width of 1.5 m/z, an activation Q value of 0.3–0.4, an activation amplitude between 30 and 39% and an activation time of 30 ms. MS^3 was used with the following parameters, activation Q value, 0.25–0.3; activation amplitude, 20–30% and activation time, 30 ms. Full-scan of product ions was acquired scanning from m/z 50 to 300 in centroid mode using a maximum injection time of 200 ms and performing 1 μscan.

3. Results and discussion

3.1. CE–MS

The ESI-MS instrumental parameters, such as capillary positioning, sheath liquid composition and flow-rate and sheath gas flow-rate were combined to optimize sensitivity and stability. A solution of the 18 chlorophenols in the running buffer was infused at pressure of 0.5 p.s.i. and applying 20 kV. During optimization, the $[M-H]^-$ ion was monitored for chlorophenols. In a first step, 30 mM DEM at pH 7.25 was selected as carrier electrolyte for the separation of chlorophenols [18]. Nevertheless, the current obtained with 30 mM DEM was around 100 μA which is too high to allow the coupling to MS owing to the discharge effects in the ESI source and the high Joule effect generated in the non-refrigerated zone between the CE and the ESI source. Thus, the concentration of the buffer was decreased in order to have lower intensity currents and 5 mM was found to be suitable, since it provided an intensity current of 12 μA and an acceptable CE resolution for most chlorophenols. The main drawback was that the separation between 4CP and 3CP was worse than that at 30 mM DEM and could not be solved even in MS because both compounds showed the same m/z. Fig. 1 shows the electropherogram recorded in full scan in the range m/z 100–300.

Make-up solution (sheath liquid) was reported to be critical to the performance of the CE–MS system. The sheath liquid has two functions, to bring the CE flow to a level suitable for ESI and to produce electrical contact between the CE eluent and the probe tip. The sheath liquid also allows changes in post-capillary solution chemistry to improve ESI characteristics and ionization efficiency [20], for instance the addition of an organic solvent improves

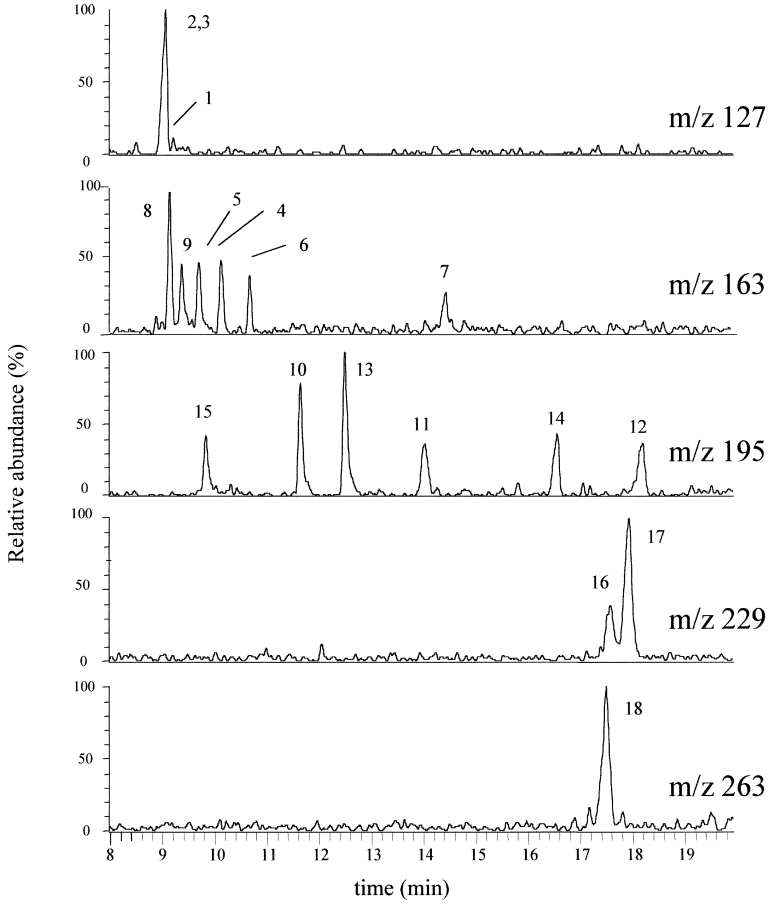

Fig. 1. CE–MS separation of a standard solution of 18 chlorophenols (2–20 μg ml^{-1} individual) using 5 m*M* diethylmalonic acid, pH 7.25 as carrier electrolyte. Experimental conditions: fused-silica capillary with a separation length of 100 cm; temperature, 25°C; voltage, 20 kV; hydrodynamic injection, 4 s. Sheath liquid: isopropanol–250 m*M* dimethylamine (80:20) at 5 μl min^{-1}. MS conditions as described in the Experimental section. Peak identification as in Table 1.

ionic evaporation. Isopropanol was chosen as it produces a more stable signal than methanol or ethanol in the negative ionization mode [19]. The sheath liquid could also be helpful in the ionization of phenols in the aqueous phase since some chlorophenols (see pK_a values in Table 1) are not totally ionized in the running buffer at the working pH 7.25. Several sheath liquid compositions were tested: isopropanol–water (80:20), isopropanol–ammonia (5%) (80:20) and isopropanol–dimethylamine (100 m*M*) (80:20) and, as in LC–ESI-MS [21], dimethylamine provided higher S/N than the other solutions, especially for the less acidic chlorophenols (2CP, 3CP, 4CP and 34DCP, see Table 1 for pK_a values) because it favors the preformation of phenolate ions in solution. This effect was less marked for the more acidic compounds, which appeared at the end of the electropherogram (2346TeCP, 2356TeCP and PCP). The concentration of dimethylamine in the sheath liquid was also optimized. The variation of the response of the $[M-H]^-$ ion vs. the concentration of dimethylamine in the sheath liquid for some chlorophenols, which shows a clear increase for dimethylamine concentrations up to 250 m*M*, is given in Fig. 2. Thus, the optimum sheath liquid composition was set at isopropanol–250 m*M* dimethylamine (80:20). Finally, the sheath liquid flow-rate ranged between 3 and 12 μl min^{-1} and, since

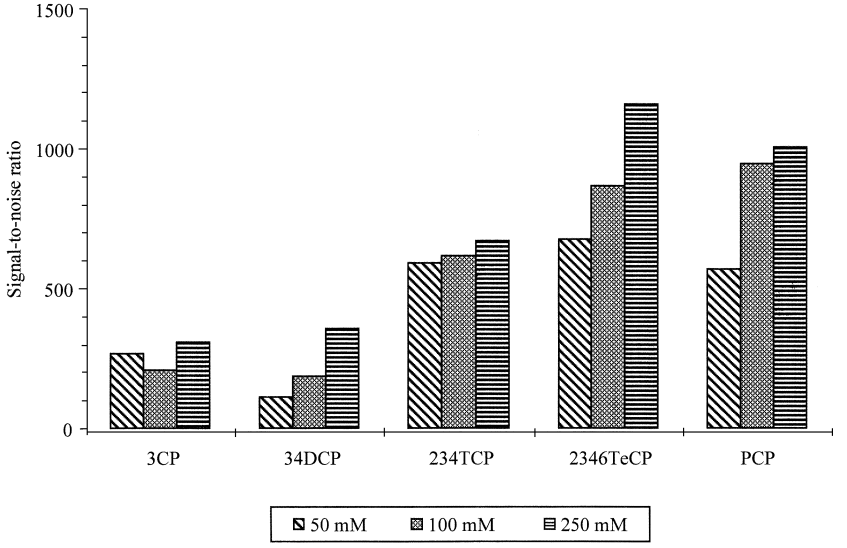

Fig. 2. Effect of dimethylamine concentration in the sheath liquid (isopropanol–dimethylamine, 80:20) on the signal-to-noise ratio of the $[M-H]^-$ ion of some chlorophenols. Abbreviations as in Table 1.

the most satisfactory results were obtained between the 5 and 10 μl min^{-1} range, 5 μl min^{-1} was chosen.

The distance that the CE capillary protrudes from the sheath liquid tube may seriously affect the performance of the system [19,20]. Here, this distance was varied between 0.1 and 0.5 mm and the highest sensitivity for the $[M-H]^-$ ion was obtained with the capillary protruding 0.2 mm beyond the electrospray needle. Nebulizing gas is also critical for the CE–MS coupling. The effect of the sheath gas flow-rate is shown in Fig. 3, where the electropherograms for a standard solution (50 μg ml^{-1}, injection time 4 s) at different sheath gas flow-rates (between 11 and 17.5 l h^{-1}) are shown. Low sheath gas flow-rates produced distorted peaks for tetrachlorophenols (peaks 16 and 17) and pentachlorophenol (peak 18) and high values provided lower resolution among dichlorophenol and trichlorophenol isomers. Moreover, an increase in the sheath gas flow-rate resulted in lower migration times. As a result, a value of 15 l h^{-1} was chosen as a compromise between peak shape and resolution.

Chlorophenols hardly showed fragmentation under negative ESI-MS conditions. The deprotonated ion dominated the spectra in all the cases (Table 1). The main drawback of using diethylmalonic acid as carrier electrolyte is that its M_r (160) is very close to that of dichlorophenols (M_r 162) and so dichlorophenols show considerable background and higher detection limits than mono-, tri-, tetra- and pentachlorophenols. To avoid interference from diethylmalonic acid for dichlorophenols, other carrier electrolytes such as L-cysteic acid (M_r 187) and ammonium acetate (M_r 59) were tested for the CE–MS coupling. Since there was high noise, the use of these electrolytes was discarded. Thus, the $[M-H]^-$ ion from the isotopic peak (^{37}Cl) was used for dichlorophenols (m/z 163).

In MS–MS studies using an ion-trap analyzer, the activation Q parameter indicates the appropriate radio frequency (RF) at which to fragment the ions. This parameter is absolutely necessary to obtain MS–MS spectra for compounds showing low M_r values, i.e., lower than 200. In general, low activation Q results in low fragmentation and low m/z fragment ions. High activation Q results in high fragmentation, but low m/z ions are not stable in the ion-trap and cannot be scanned out. Two other parameters were controlled: the activation amplitude and the activation time. The former is the amplitude of the resonance excitation RF voltage, which determines the kinetic energy of ions in the mass analyzer and the latter is the duration in milliseconds

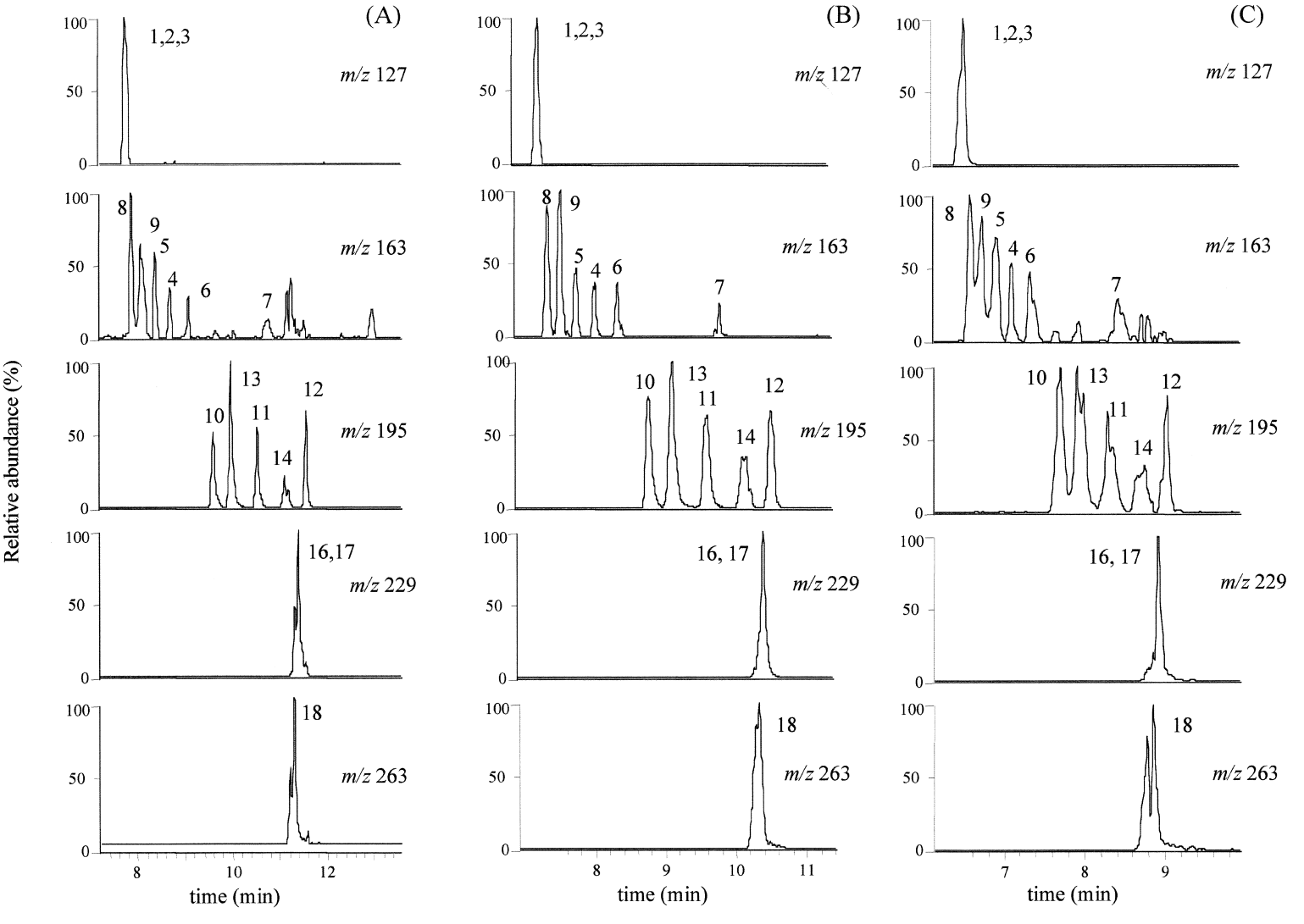

Fig. 3. Effect of sheath gas flow-rate on the CE–MS separation of chlorophenols. (A) 11 l h^{-1}; (B) 15 l h^{-1}; (C) 17.5 l h^{-1}. Experimental conditions: fused-silica capillary with a separation length of 80 cm; temperature, 25°C; voltage, 20 kV; hydrodynamic injection, 4 s. MS conditions as in the Experimental section. Peak identification as in Table 1.

of the RF voltage. In general, high activation amplitudes and long activation times resulted in high fragmentation. In our case, for MS–MS an activation amplitude of 30%, an activation time of 30 ms and a *Q* value of 0.3 were used for mono-, di- and trichlorophenols. Monochlorophenols presented major problems due to low stabilisation of the fragment ions whereas di- and trichlorophenols showed stable signals in MS–MS. For 2,4,6-trichlorophenol, the activation amplitude had to be increased to 39% (Fig. 4). For tetrachlorophenols and pentachlorophenol, *Q* was increased to 0.5. Only 2,3,5,6-tetrachlorophenol gave a stable signal while 2,3,4,6-tetrachlorophenol presented a very unstable

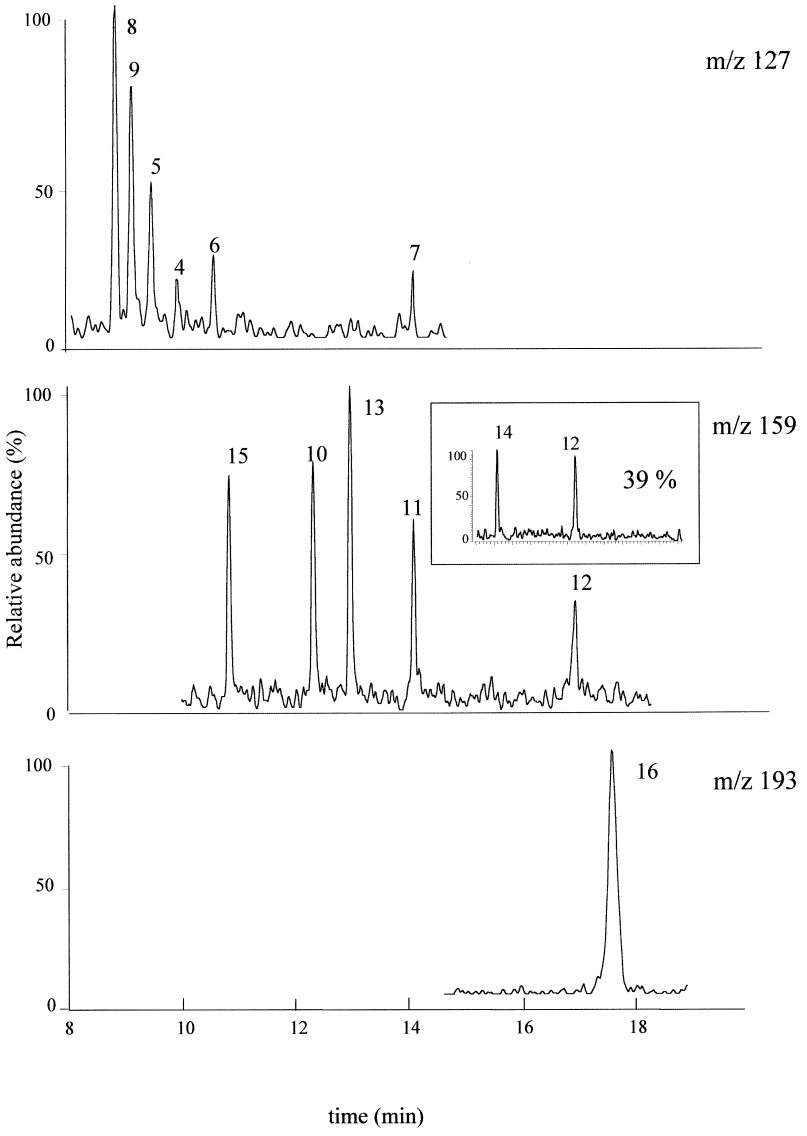

Fig. 4. CE–MS–MS of a standard solution of chlorophenols (2–20 μg ml^{-1}, hydrodynamic injection 4 s). MS–MS parameters: activation amplitude: 30% for all the compounds except for 2,4,6-trichlorophenol (peak 12) 39%, activation *Q* 0.3, activation time 30 ms. Experimental conditions: fused-silica capillary with a separation length of 80 cm; temperature 25°C; voltage, 20 kV. Other MS conditions as in the Experimental section. Peak identification as in Table 1.

fragmentation pattern, and so the MS–MS spectra could not be obtained. Fragmentation of pentachlorophenol was never possible. Table 1 shows the product ions obtained in ESI-MS–MS and ESI-MS^3 by infusion of a standard solution of phenols (50 μg ml^{-1}) in running buffer. Since consecutive losses of HCl units were observed, MS–MS can also be used to obtain additional information about the analytes in the product-ion scan mode, providing a more accurate identification.

Detection limits for standard solutions ($S/N=3$) using CE–diode array detection (DAD), CE–MS and CE–MS–MS are given in Table 2. Values between 0.3 and 2 μg ml^{-1} in CE–DAD and between 0.5 and 10 μg ml^{-1} in CE–MS were obtained. Detection limits for chlorophenols with low chlorination (mono- and dichlorophenols) using CE–MS are considerably higher than those using CE–DAD. In contrast, for highly chlorinated compounds, the detection limits are more similar or even lower in CE–MS. This fact has also been observed using LC–atmospheric pressure chemical ionization (APCI) MS [22], which provided higher sensitivity for tetra- and pentachlorophenols. The advantage of CE–MS over CE–DAD is that the identification is based on the mass spectral characterization rather than on migration times. As for CE–MS–MS a compromise between sensitivity and fragmentation should be taken into account when choosing the optimum MS–MS conditions. The activation amplitude should be chosen carefully because high values provided fragmentation of the precursor ion but a decrease in trapping efficiency occurred and as a result, the sensitivity is reduced. This can explain why the limits of detection for MS–MS were slightly higher than for MS. For instance, the detection limit of 2,4,6-trichlorophenol (see Table 2) is five-times higher in MS–MS than in MS which can be related to the relatively high activation amplitude (39%) needed.

Table 2
Limits of detection (μg ml^{-1}) for standard solutions using CE–DAD, CE–MS and CE–MS–MS[a]

Compound	Limit of detection (μg ml^{-1})		
	CE–DAD[b]	CE–MS	CE–MS–MS
2CP	0.5	10.0	–[c]
3CP	2.0	5.0	–[c]
4CP	1.0	5.0	–[c]
23DCP	1.0	5.0	10.0
24DCP	0.5	5.0	3.0
25DCP	1.0	5.0	10.0
26DCP	0.5	5.0	10.0
34DCP	0.5	2.5	1.0
35DCP	0.3	5.0	0.7
234TCP	0.5	1.0	2.0
235TCP	0.5	1.0	2.0
236TCP	1.0	2.0	6.0
245TCP	0.5	0.7	3.0
246TCP	0.5	2.0	10.0
345TCP	ND	1.0	1.5
2346TeCP	1	0.5	0.7
2356TeCP	1	0.5	–[c]
PCP	1	1.0	–[d]

[a] Experimental conditions as in text.
[b] 30 m*M* diethylmalonic acid, pH 7.25, hydrodynamic injection, 4 s [18].
[c] Unstable product ions.
[d] Not fragmented at all.
ND: Not determined.

4. Conclusions

CE–MS and CE–MS–MS were applied to analyze 18 positional isomers of chlorophenols using an ion-trap mass spectrometer. To couple CE to MS, several parameters have been optimized. First, the concentration of carrier electrolyte was decreased in order to avoid discharge effects in the source and to minimize the Joule effect. The optimum conditions were 5 m*M* diethylmalonic acid at pH 7.25. The sheath gas flow-rate and sheath liquid composition were also relevant on the performance of the system. Dimethylamine was added to the sheath liquid to favor the preformation of phenolate ions in the solution. The optimum composition for the sheath liquid was: isopropanol–250 m*M* dimethylamine (80:20). MS–MS parameters such as activation amplitude, activation *Q* and activation time have also been studied in order to obtain fragmentation with an acceptable sensitivity. Low values of activation *Q* were needed, between 0.3 and 0.5, to stabilize phenolate ions. In CE–MS, $[M-H]^-$ was the diagnostic ion for all the chlorophenols. In CE–MS–MS, the $[M-H-HCl]^-$ ion was observed for mono-, di-, tri- and tetrachlorophenols, whereas no fragmentation occurred for pentachlorophenol. As chloro-

phenols are relatively stable under MS–MS conditions, a high energy (up to 39%) was needed for fragmentation and high detection limits were obtained because the parent ion was partially scanned out of the trap and the signal-to-noise ratio decreased.

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