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Capillary electrophoresis–mass spectrometry for the analysis of quaternary ammonium herbicides

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Abstract

Conditions for the simultaneous determination of the three herbicides paraquat, diquat and difenzoquat and the two plant growth regulators chlormequat and mepiquat by pressure-assisted capillary electrophoresis coupled to mass spectrometry (ion-trap) using electrospray as ionisation source have been established. A 200 m*M* formic acid–ammonium formate buffer solution at pH 3.0 with 50% of methanol was used as carrier electrolyte. Some capillary electrophoresis–mass spectrometry parameters such as sheath liquid and sheath gas flow-rates, sheath liquid composition, electrospray voltage and the CE capillary position were optimised. The MS and MS–MS spectra of positive ions were studied in order to obtain structural information for the confirmation of the identity. The use of labelled standards allowed to confirm fragment ions assignation. The detection limits, based on a signal-to-noise ratio of 3:1, were between 0.5 and 2.5 mg l^{-1} with hydrodynamic injection (10 s) and between 1 and 10 μg l^{-1} with electrokinetic injection (20 s, 10 kV) using standards in ultrapure water. Quality parameters such as linearity and run-to-run precision ($n=6$) were established. Quantitation was carried out using labelled standards. The method has been applied to the analysis of contaminated irrigation water and spiked mineral water samples.

Keywords: Water analysis; Quaternary ammonium herbicides; Pesticides

1. Introduction

The increased dependency of modern agricultural practice upon herbicides to control weeds and increase crop yield has led to concerns about their residues in different environmental samples. Among pesticides, the group of quaternary ammonium herbicides (quats) is of major economic importance. This group includes two non-selective contact herbicides, paraquat (PQ), which has been marketed in over 130 countries as highly effective herbicide, and diquat (DQ), the selective herbicide difenzoquat (DF), and two plant growth regulators, chlormequat (CQ) and mepiquat (MQ) [1]. These compounds are widely used to control many types of weeds, insects and other pests in a wide variety of agricultural and non-agricultural settings. Because of their moderate toxicity [2], there is a great concern about the presence of these compounds in fruits, soils and waters. For drinking waters, the Office of Water of the US Environmental Protection Agency (EPA) has established a maximum contaminant level of 20 μg l^{-1} for DQ and a maximum contaminant level goal of 3 μg l^{-1} for PQ [3,4]. Thus, analytical techniques of enough sensitivity and selectivity are needed to monitor the quality of drinking water.

Although a great number of techniques have been used for the analysis of these compounds, ion-pair

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high-performance liquid chromatography (HPLC) [5–7] and capillary electrophoresis (CE) [5,8–12] using UV detection were the most frequently applied. However, the analysis of the plant growth regulators, which do not have chromophore groups, requires the use of indirect UV detection [13]. The major disadvantage of these methods is the lack of analytical specificity, which results in identification and quantification difficulties. The use of mass spectrometry (MS) as a detection technique allows to overcome these problems and permits the simultaneous analysis of the three herbicides and the two plant growth regulators. Recently, there is a great interest about the presence of residues of chlormequat in fruits and different studies applying liquid chromatography coupled to mass spectrometry (LC–MS) have been published [14–17]. LC–MS has also been widely applied for the analysis of quats in drinking water [18–22].

Since quats are ionic species the use of CE for their analysis without the requirement of using ion-pair reagents seems ideal. Recently, the development in coupling CE to mass spectrometry (CE–MS) has been remarkable. This coupling combines the advantages of both techniques such as the high resolution power of CE and the detection and identification potential of MS, which provides a powerful tool for the separation and identification of quats. Among the different ionisation sources developed for LC–MS, the electrospray (ES) is the most widely used for on-line CE–MS [23–25]. Nevertheless, when coupling the CE to the mass spectrometer an interface is necessary to close the electrical circuit and to achieve the flow needed (about $\mu l\ min^{-1}$) for electrospray optimal conditions. The most frequently used interface is a coaxial sheath flow system [26], although now, with developments in nanoelectrospray techniques, new interfaces based in sheathless systems are also used [27]. Few papers using CE–MS for the analysis of quats [28–30] have been published and only one proposes a method for the simultaneous analysis of the five compounds [28].

In this work the potential of CE–MS using electrospray as an ionisation source for the analysis of quats in the environment was studied. Different coupling parameters such as sheath liquid and sheath gas flow-rates, sheath liquid composition, electrospray voltage, and capillary position into the electrospray source were optimised. The MS–MS spectra of positive ions were studied in order to identify the compounds and the fragments obtained were discussed. Quality parameters such as limits of detection (LODs), run-to-run precision and linearity were obtained using both hydrodynamic and electrokinetic injection. Quantitation using conventional internal standards and labelled compounds was compared. Finally the method was applied to the analysis of a highly contaminated irrigation water and spiked mineral water samples.

2. Experimental

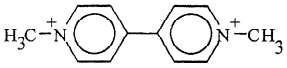
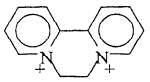
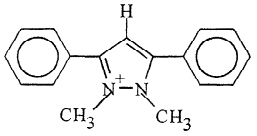
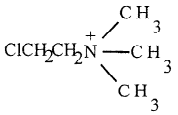
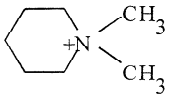
2.1. Chemicals

The quaternary ammonium herbicides were obtained from the following sources: diquat (1,1′-diethylene-2,2′-bipyridinium ion, DQ), difenzoquat (1,2-dimethyl-3,5-diphenylpyrazolium ion, DF) and chlormequat (2-chloroethyltrimethylammonium ion, CQ) were purchased from Chemservice (West Chester, PA, USA), paraquat (1,1′-dimethyl-4,4′-bipyridinium ion, PQ) from Sigma (St. Louis, MO, USA), and mepiquat (1,1′-dimethylpyperidinium ion, MQ) from Riedel-de Haën (Seelze, Germany). Ethylviologen (1,1′-diethyl-4,4′-bipyridinium ion, EV) purchased from Aldrich (Milwaukee, WI, USA) and heptylviologen (1,1′-diheptyl-4,4′-bipyridinium ion, HV) from TCI (Tokyo, Japan) were used as internal standards. The deuterated analytes paraquat-d_8 (1,1′-dimethyl-4,4′-bipyridinium-rings-d_8 ion, PQ-d_8), diquat-d_4 (1,1′-ethylene-d_4-2,2′-dipyridinium ion, DQ-d_4) and chlormequat-d_4 (2-chloroethyl-d_4-trimethylammonium ion, CQ-d_4, 100 $mg\ l^{-1}$) were obtained from Dr. Ehrenstorfer (Augsburg, Germany). The structures of all these compounds are shown in Tables 1 and 2.

All the reagents were of analytical grade. Methanol, acetic acid (100%), formic acid (98–100%), ammonium acetate, sodium hydroxide and hydrochloric acid (25%) were purchased from Merck (Darmstadt, Germany), and ammonium formate from Fluka (Buchs, Switzerland). Water was purified using an Elix 3 coupled to a Milli-Q system (Millipore, Bedford, MA, USA).

Stock standard solutions of individual quats, PQ-

Table 1
Mass spectra of quaternary ammonium herbicides in CE–MS and CE–MS–MS

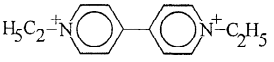
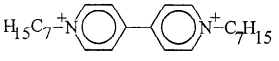
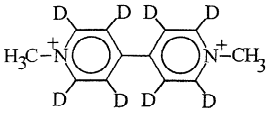
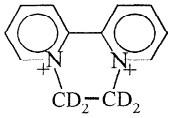
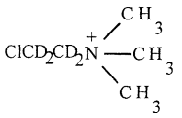
Structure	Name	Retention time (min)	MS spectra		MS–MS spectra		
			m/z (% relative abundance)	Assignation	Precursor ion *m/z*	Product ion *m/z* (% relative abundance)	Assignation
	Paraquat (PQ)	10.4	186 (100) 185 (22) 171 (28) 93 (24)	$[Cat]^{+\cdot}$ $[Cat-H]^{+}$ $[Cat-CH_3]^{+}$ $[Cat]^{2+}$	186	185 (<5) 171 (100)	$[Cat-H]^{+}$ $[Cat-CH_3]^{+}$
	Diquat (DQ)	10.8	184 (100) 183 (57) 92 (12)	$[Cat]^{+\cdot}$ $[Cat-H]^{+}$ $[Cat]^{2+}$	184	183 (96) 168 (100) 158 (71) 157 (92)	$[Cat-H]^{+}$ $[Cat-NH_2]^{+}$ or $[Cat-CH_4]^{+\cdot}$ $[Cat-C_2H_2]^{+\cdot}$ $[Cat-C_2H_3]^{+}$ or $[Cat-CNH]^{+\cdot}$
	Difenzoquat (DF)	16.9	249 (100)	$[Cat]^{+}$	249	234 (25) 208 (100) 193 (83) 146 (27) 131 (33) 118 (6)	$[Cat-CH_3]^{+\cdot}$ $[Cat-(NCH_3)_2+2H+CH_3]^{+\cdot}$ $[Cat-(NCH_3)_2+2H]^{+}$ $[Cat-C_6H_5-CNCH_3+CH_3]^{+}$ $[Cat-C_6H_5-CNCH_3]^{+\cdot}$ $[Cat-C_6H_5-C_2HNCH_3]^{+}$
	Chlormequat (CQ)	12.5	122 (100) 124 (34)	$[Cat]^{+}$ $[Cat+2]^{+}$	122	94 (36) 86 (7) 63 (28) 59 (65) 58 (100)	$[Cat-C_2H_4]^{+}$ $[Cat-HCl]^{+}$ $[Cat-N(CH_3)_3]^{+}$ $[Cat-ClC_2H_4]^{+\cdot}$ $[Cat-ClC_2H_5]^{+}$
	Mepiquat (MQ)	12.4	114 (100)	$[Cat]^{+}$	114	99 (82) 98 (100) 58 (96)	$[Cat-CH_3]^{+}$ $[Cat-CH_3-H]^{+}$ $[Cat-C_4H_8]^{+}$

d_8, DQ-d_4 and internal standards (1 mg ml^{-1}) were prepared in ultrapure water and stored in plastic vials to prevent adsorption. Working solutions were obtained by dilution with ultrapure water, and were filtered through a 0.45 μm nylon filter. Buffers were prepared from an aqueous solution of formic acid 400 m*M* and the pH 3.0 was adjusted with ammonium formate (400 m*M*). The final carrier electrolyte was obtained by mixing the previous buffer with methanol 1:1.

2.2. Capillary electrophoresis instrumentation

The experiments were performed on a Beckman P/ACE 5500 capillary electrophoresis system (Fullerton, CA, USA) equipped with a diode array

Table 2
Mass spectra of internal standard and labelled compounds in CE–MS and CE–MS–MS

Structure	Name	Retention time (min)	MS spectra		MS–MS spectra		
			m/z (% relative abundance)	Assignation	Precursor ion *m/z*	Product ion *m/z* (% relative abundance)	Assignation
	Ethylviologen (EV)	11.5	214 (100) 213 (11) 185 (45) 107 (26)	$[Cat]^{+\cdot}$ $[Cat-H]^{+}$ $[Cat-C_2H_5]^{+}$ $[Cat]^{2+}$	214	213 (22) 185 (100)	$[Cat-H]^{+}$ $[Cat-C_2H_5]^{+}$
	Heptylviologen (HV)	14.2	354 (100) 353 (12) 255 (61) 177 (85)	$[Cat]^{+\cdot}$ $[Cat-H]^{+}$ $[Cat-C_7H_{15}]^{+}$ $[Cat]^{2+}$	354	353 (<5) 255 (100)	$[Cat-H]^{+}$ $[Cat-C_7H_{15}]^{+}$
	Paraquat-d_8 (PQ-d_8)	10.4	194 (100) 193 (9) 179 (15) 97 (13)	$[Cat]^{+\cdot}$ $[Cat-H]^{+}$ $[Cat-CH_3]^{+}$ $[Cat]^{2+}$	194	193 (45) 179 (100)	$[Cat-H]^{+}$ $[Cat-CH_3]^{+}$
	Diquat-d_4 (DQ-d_4)	10.8	188 (100) 187 (34) 186 (67) 94 (13)	$[Cat]^{+\cdot}$ $[Cat-H]^{+}$ $[Cat-D]^{+}$ $[Cat]^{2+}$	188	187 (35) 186 (10) 171 (26) 170 (33) 169 (100) 160 (15) 159 (26)	$[Cat-H]^{+}$ $[Cat-D]^{+}$ $[Cat-NHD]^{+}$ $[Cat-ND_2]^{+}$ $[Cat-CD_3H]^{+\cdot}$ $[Cat-C_2D_2]^{+\cdot}$ or $[Cat-CND]^{+\cdot}$ $[Cat-C_2D_2H]^{+}$
	Chlormequat-d_4 (CQ-d_4)	12.5	126 (100) 128 (34)	$[Cat]^{+}$ $[Cat+2]^{+}$	126	94 (68) 89 (5) 67 (21) 59 (71) 58 (100)	$[Cat-C_2D_4]^{+}$ $[Cat-DCl]^{+}$ $[Cat-N(CH_3)_3]^{+}$ $[Cat-ClC_2D_4]^{+\cdot}$ $[Cat-ClC_2D_4H]^{+}$

detection system and modified to permit the coupling with a mass spectrometer. The electrophoretic separations were carried out using uncoated fused-silica capillaries (Beckman) with a total length of 80 cm× 50 μm I.D. and a 200 m*M* formic acid–ammonium formate buffer solution, pH 3.0, containing 50% of methanol as carrier electrolyte. The temperature was held at 25 °C. The buffer was filtered through a 0.45 μm membrane filter, and degassed by sonication before use. Samples were loaded by using two injection modes: hydrodynamic injection pressure assisted (35 kPa, 10 s) and electrokinetic injection (+10 kV, 10–20 s). When the injection was performed, the electrospray voltage was turned off in

order to prevent the electrokinetic introduction of the analytes. Pressure-assisted electrophoretic separations were performed by applying simultaneously a voltage of +25 kV and an overimposed pressure of 35 kPa on the inlet vial during the whole run. The CE instrument was controlled using a Beckman P/ACE station software version 1.0.

2.3. Mass spectrometry instrumentation

An LCQ mass spectrometer (Finnigan, San Jose, CA, USA) equipped with a tricoaxial pneumatically assisted ES ionisation source designed for the CE–MS coupling and with an ion trap as analyser was used. A solution of methanol–10 m*M* acetic acid (9:1) at a flow-rate of 3 μl min^{-1} was used as sheath liquid after degassed by sonication in order to avoid bubble formation. The ES was pneumatically assisted by nitrogen as sheath gas at a flow-rate of 13.5 l h^{-1} (15 arbitrary unities). The electrospray needle was set at +4.0 kV and the heated capillary temperature was held at 250 °C. The CE capillary protrudes from the electrospray needle 0.1 mm, and the distance to the heated capillary was 1.5 cm. Moreover 0.5–1 cm of the polyamide coating was eliminated from the end of the fused-silica capillary in order to improve the wetability by the sheath liquid.

CE–MS data acquisition was carried out in full scan mode from m/z 50 to 400 in centroid mode using a maximum injection time of 100 ms and performing 6 μscans. The CE–MS–MS data acquisition was performed in the full scan mode using a maximum injection time of 200 ms with 1 μscans. Isolation width (m/z) between 1.2 and 1.5 was used, the activation Q (AQ) was set between 0.3 and 0.4, the activation amplitude (AA, %) from 28 to 40 and the activation time (AT) between 32 and 35 ms. Mass spectrometry data were processed with a Xcalibur 1.2 software.

2.4. Capillary conditioning

New capillaries were pre-treated using 0.1 *M* hydrochloric acid for 15 min, ultrapure water for 15 min, 1 *M* sodium hydroxide for 30 min, and finally rinsed with ultrapure water for 30 min. At the beginning of each session, the capillary was rinsed with 400 m*M* formic acid–ammonium formate buffer at pH 3.0 during 30 min and finally with the carrier electrolyte for 60 min. The conditioning method was carried out daily in order to prevent adsorption of the quaternary ammonium herbicides on the capillary wall. Finally, the capillary was rinsed with carrier electrolyte for 1 min between runs and stored after rinsed with 50 m*M* formic acid–ammonium formate buffer at pH 3.0.

3. Results and discussion

3.1. Preliminary study and electrophoretic separation

In this work a volatile buffer, 50 m*M* acetic acid–ammonium acetate at pH 4.0 with 10% methanol, was used as carrier electrolyte. At these conditions, poor resolution for PQ/DQ and CQ/MQ was obtained. Moreover, an important problem in the analysis of quats by MS is the decrease in the responses of these compounds when coelution between them occurred due to ionic suppression in the electrospray source [33]. For this reason the electrophoretic separation of quats must be improved.

In capillary electrophoresis the poor resolution for the quats is mainly due to the important adsorption on the wall silica capillary that produced tailing peaks. To improve the separation different parameters such as buffer concentration, amount of methanol and pH of carrier electrolyte were studied. An increase in the ionic strength of the carrier electrolyte by increasing the buffer concentration reduced the adsorption of these compounds and improved the peak shapes. Different buffer concentrations from 50 to 260 m*M* were tested and 200 m*M* was chosen as a compromise between separation and the capillary current suitable for the CE–MS coupling. Moreover, the addition of different amounts of methanol to the carrier electrolyte was also studied in order to achieve better separations. It was observed that the increase of methanol improved the resolution between PQ and DQ but decreased the resolution between CQ and MQ. For instance, using a 200 m*M* acetic acid–ammonium acetate at pH 4.0 with 30% methanol, a baseline resolution for PQ/DQ was obtained but, in contrast, CQ/MQ comigrated. With higher amounts of methanol, the migration order of

MQ and CQ changed due to the higher mobility of MQ and using 50% of methanol a base line resolution for these compounds was obtained. So, to get a good electrophoretic separation of quats, high concentration buffers with high amounts of methanol were required although the analysis time increased because of the lower electroosmotic flow. Moreover, buffers at lower pH (3.5 and 3.0) were tested in order to reduce the ionisation of silanol groups of the capillary wall and consequently to decrease the electrostatic interaction between quats and the negatively charged surface. A 200 m*M* formic acid–ammonium formate buffer at pH 3.0 with 50% of methanol used as carrier electrolyte gave the best electrophoretic separation. Nevertheless, in these conditions the electroosmotic flow was too low to guaranty the introduction of the ions into the mass spectrometer and keep the electric contact. For this reason, an overimposed pressure of 35 kPa on the inlet vial was applied during the whole run. Migration times of quats, internal standards and labelled compounds under optimal electrophoretic conditions are given in Tables 1 and 2. Good electrophoretic separation of all the compounds was obtained.

3.2. Optimisation of the coupling parameters

The ES-MS instrumental parameters such as sheath liquid flow-rate, sheath gas flow-rate, sheath liquid composition, electrospray voltage, size of the CE capillary protruding from the sheath liquid tube and distance between the electrospray needle and the MS heated capillary were optimised in order to obtain the higher response. To optimise these parameters a standard solution of 15 mg l^{-1} of quats prepared in ultrapure water was used. The introduction of the standard solution was performed by hydrodynamic injection (10 s, 35 kPa).

Fig. 1 shows the normalised response of the five herbicides with the variation of the sheath liquid flow-rate from 3 to 12 μl min^{-1} (Fig. 1a), the sheath gas flow-rate from 4.5 to 18 l h^{-1} (Fig. 1b) and the amount of methanol (from 50 to 90%) as organic solvent into the sheath liquid (Fig. 1c). When the sheath liquid flow-rate increased (Fig. 1a) the response of the herbicides decreased until 5 μl min^{-1} due to the dilution. Moreover, a maximum response of the herbicides was obtained when 13.5 l h^{-1} sheath gas flow-rate was used (Fig. 1b). At flow-rates too low or too high the response decreased due to the electrospray unstability. In order to obtain the maximal response sheath liquid and sheath gas flow-rates of 3 μl min^{-1} and 13.5 l h^{-1}, respectively, were used.

The composition of the sheath liquid is critical to the performance of the CE–MS system. Methanol was added to increase the ionisation efficiency and acetic acid was chosen as electrolyte to have a conductive solution that permits closing the electrical circuit between the CE and the electrospray source. The normalised response of the herbicides with the amount of methanol in the sheath liquid is given in Fig. 1c. The maximal response was obtained using a mixture of methanol–10 m*M* acetic acid (9:1) as sheath liquid.

The electrospray voltage was optimised from 3.0 to 4.5 kV. The response increased when the electrospray voltage raised but when values higher than 4.0 kV were used discharges occurred in the electrospray source. So 4.0 kV was chosen as the optimum value. The distance that the CE capillary protrudes from the electrospray needle was also optimised because it may seriously affect the performance of the system [23]. The variation of the normalised response of the herbicides when this distance was varied between 0.05 to 0.3 mm is shown in Fig. 1d. Maximum response was achieved when the CE capillary only protruded 0.1 mm from the sheath liquid tube. At lower and higher values the response decreased probably due to the unstability in the formation of the charged droplets in the electrospray.

The distance between the electrospray needle and the MS heated capillary must be optimised in order to achieve a high efficiency in the transmission of the ions. This parameter was studied by positioning the interface in the minor and in the major values permitted by the instrument, 1.5 and 2.5 cm, respectively. An important increase in the response (60–90%) was observed when 1.5 cm was used, due to the proximity between the spray and the MS heated capillary which permits the introduction of a major number of ions into the mass spectrometer.

3.3. Mass spectrometry

Full scan MS and MS–MS spectra were obtained for the cationic herbicides in order to identify and

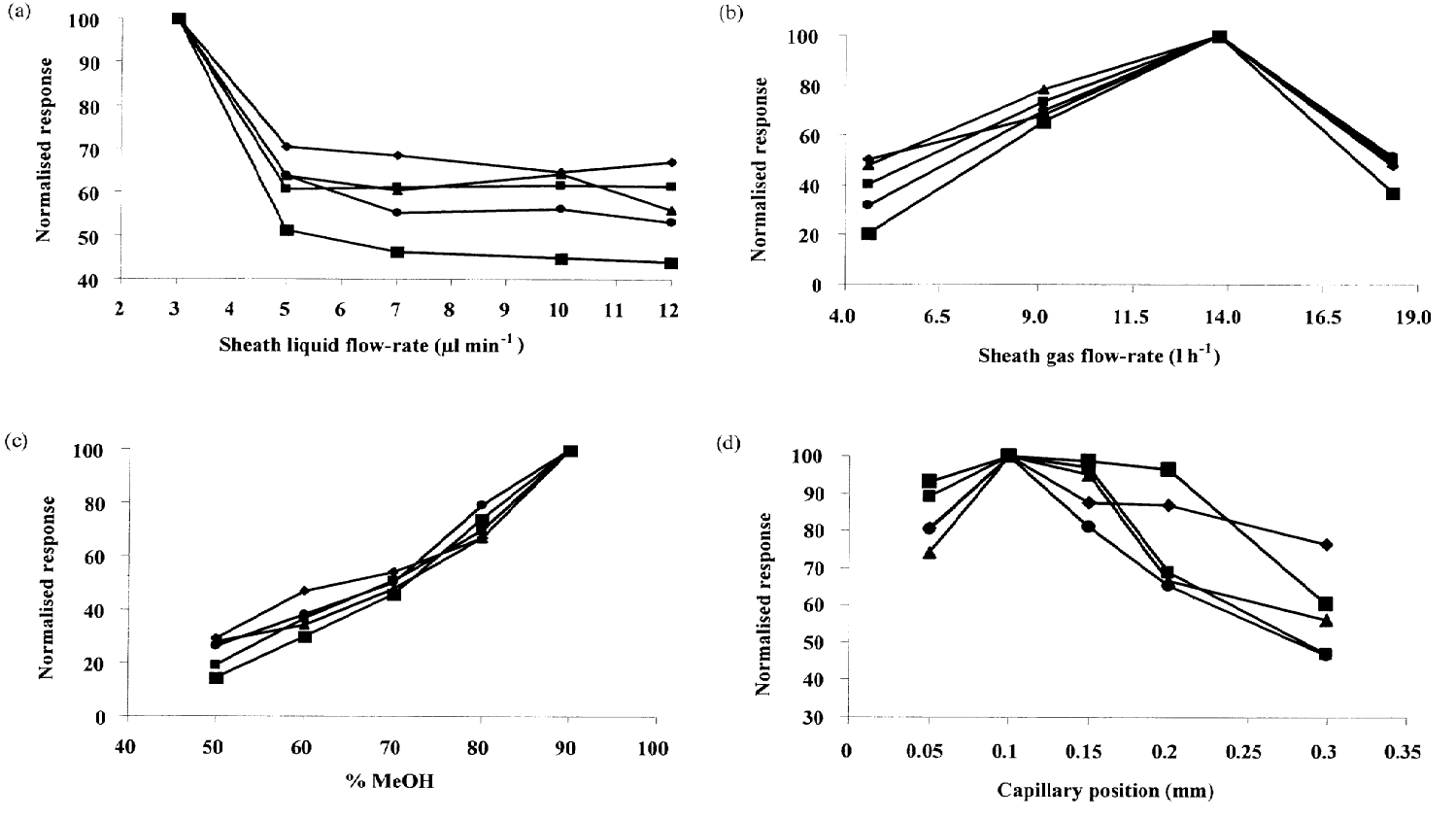


Fig. 1. Normalised response of quaternary ammonium herbicides. Effect of: (a) sheath liquid flow-rate, (sheath gas flow-rate, 13.5 l h^{-1}; sheath liquid, methanol–10 m*M* acetic acid, 8:2); (b) sheath gas flow-rate, (sheath liquid flow-rate, 3 μl min^{-1}; sheath liquid, methanol–10 m*M* acetic acid, 8:2); (c) amount of methanol in the sheath liquid (sheath gas flow-rate, 13.5 l h^{-1}; sheath liquid flow-rate, 3 μl min^{-1}) and (d) CE capillary distance that protrudes from the electrospray needle. Electrophoretic conditions: Standard solution (15 mg l^{-1}); hydrodynamic injection, 10 s (35 kPa); applied potential, +25 kV with an overimposed pressure of 35 kPa on the inlet vial during the whole run; carrier electrolyte 200 m*M* formic acid–ammonium formate containing (pH 3.0) 50% methanol. ♦, PQ; ▲, DQ; ■, CQ; ●, MQ; ■, DF.

characterise these compounds. The CE–MS spectra data for quats, labelled compounds and internal standards are given in Tables 1 and 2. DF, CQ and MQ gave the molecular ion $[Cat]^+$ at m/z 249, 122 and 114, respectively, as the most abundant peak. CQ also gave the ion at m/z 124 corresponding to the ^{37}Cl isotopic contribution and CQ-d_4 (Table 2) showed the equivalent spectrum to CQ shifted in mass according to the deuterium atoms (m/z 126 and 128). These spectral data agree with those obtained in a previous work [28] using a quadrupole as analyser, except for CQ. For this compound the fragment ion at m/z 73 assigned to $[Cat\text{-}Cl\text{-}CH_2]^{+\cdot}$ was not observed when we used an ion-trap. PQ and DQ showed a more fragmented CE–MS spectra. PQ gave the molecular radical ion $[Cat]^{+\cdot}$ at m/z 186 as the most abundant peak and the ions at m/z 185 and 171 assigned to $[Cat\text{-}H]^+$ and $[Cat\text{-}CH_3]^+$, respectively. PQ-d_8 gave a similar spectrum to PQ shifted in mass according to the deuterium atoms in the aromatic rings. Moreover, the spectral data of this deuterated compound showed that the loss of H came from the methyl group. For both, DQ and DQ-d_4 the most abundant peak was the molecular radical ion $[Cat]^{+\cdot}$ (m/z 184 and 188, respectively). Moreover, the peak in the spectrum of DQ at m/z 183 assigned to $[Cat\text{-}H]^+$ correspond to two peaks in the DQ-d_4 spectrum (m/z 187 and 186). While the ion at m/z 186 could be assigned to the loss of a deuterium atom from the ethylene group, the ion at m/z 187 could only be explained if an hydrogen–deuterium exchange occurred in the molecule [34], making possible the loss of H of the d_4-ethylene group. Other interesting peaks in the PQ, PQ-d_8, DQ and DQ-d_4 spectra are the doubly charged molecular ions $[Cat]^{2+}$, m/z 93, 97, 92 and 94, respectively. These doubly charged ions were also observed by other authors when using CE–MS [28–30] and LC–MS [33,35] in combination with methanol/water mobile phases or carrier electrolytes, but they are inhibited by the presence of acetonitrile or ion-pair reagents in the mobile phase [18].

The two internal standards, EV and HV, have equivalent structures to PQ and the spectra were quite similar showing the molecular radical ion $[Cat]^{+\cdot}$, the doubled charged ion $[Cat]^{2+}$, the loss of an hydrogen from the alkyl group $[Cat\text{-}H]^+$ and the loss of the alkyl group ($[Cat\text{-}C_2H_5]^+$ for EV and $[Cat\text{-}C_7H_{15}]^+$ for HV).

During the collision-induced dissociation (CID) step in an ion trap, a resonance excitation voltage has to be applied to the endcap electrodes to fragment parent ions into product ions in order to perform the MS–MS experiments. This CID energy is controlled by the magnitude (AA, activation amplitude) and the duration (AT, activation time) of the resonant excitation voltage and the magnitude of the trapping radio frequency (RF) voltage (AQ, activation Q) [31,32]. We have used the values previously optimised for quats [18] and we have obtained the corresponding values for labelled compounds and internal standards.

PQ and PQ-d_8 gave a similar MS–MS spectrum shifted in mass according to the deuterated atoms and providing as the most abundant peak the fragment corresponding to the loss of the methyl group. The lost of an hydrogen from the methyl group was also observed. DQ, CQ, MQ and DF showed a more complicated MS–MS spectra. The lost of an hydrogen was one of the more abundant peaks (m/z 183, $[Cat\text{-}H]^+$) for DQ that in the spectrum of DQ-d_4 corresponded to the peaks at m/z 187 ($[Cat\text{-}H]^+$) and 186 ($[Cat\text{-}D]^+$). The base peak for DQ was the ion at m/z 168, assigned in previous works [18,28] to $[Cat\text{-}NH_2]^+$ which can be related with peaks at m/z 171 ($[Cat\text{-}NHD]^+$) and 170 ($[Cat\text{-}ND_2]^+$) in the DQ-d_4 spectrum, taking into account the hydrogen–deuterium exchange previously commented. Moreover, the ion at m/z 168 can also be assigned to $[Cat\text{-}CH_4]^{+\cdot}$ in agreement with the ion at m/z 169 $[Cat\text{-}CD_3H]^{+\cdot}$ that is the base peak for DQ-d_4. DQ also gave two fragments at m/z 158 and 157 that can be correlated with losses on the ethylene group, $[Cat\text{-}C_2H_2]^{+\cdot}$ and $[Cat\text{-}C_2H_3]^+$ or $[Cat\text{-}CNH]^{+\cdot}$, respectively, which has been confirmed in the DQ-d_4 spectrum by the presence of fragment ions at m/z 159 and 160 assigned to $[Cat\text{-}C_2D_2H]^+$ (with an hydrogen–deuterium exchange) and $[Cat\text{-}C_2D_2]^{+\cdot}$ or $[Cat\text{-}CND]^{+\cdot}$. The lost of C_2D_3 was also observed although the relative abundance of this ion was lower than 5%.

CQ gave the ions at m/z 94, 86, 63, 59 and 58, corresponding to $[Cat\text{-}C_2H_4]^+$, $[Cat\text{-}HCl]^+$, $[Cat\text{-}N(CH_3)_3]^+$, $[Cat\text{-}ClC_2H_4]^{+\cdot}$ and $[Cat\text{-}ClC_2H_5]^+$, respectively. The fragment ions at m/z 86 and 94,

explained as the neutral loss of HCl and as an ion/molecule reaction between the ion at m/z 59 and the HCl present in the ion trap [18], respectively, were not observed with quadrupol mass spectrometers [14,15,28]. The labelled CQ-d_4 gave fragment ions that agree with those proposed for the non-labelled compound. MQ gave the fragment ions at m/z 99, 98 and 58, assigned as $[Cat\text{-}CH_3]^+$, $[Cat\text{-}CH_3\text{-}H]^+$ and $[Cat\text{-}C_4H_8]^+$ respectively. The fragment ion at m/z 99 was not observed when a quadrupol was used as analyser [28]. DF gave the ions at m/z 234, 208, 193, 146, 131 and 118 (Table 1). The ion at m/z 234 produced a radical $\cdot CH_3$ which can react by an ion-molecule reaction in the ion-trap with the ions at m/z 193 and 131 to produce the ions at m/z 208 and 146, respectively. All the ions obtained for MQ, CQ and DF with the CE–MS–MS experiments agree with those obtained with LC–MS–MS using the same analyser [18]. The MS–MS spectra of the two internal standards EV and HV were very simple and they only gave the loss of the alkyl group as the most abundant peak and also the loss of hydrogen from the alkyl group.

3.4. Quality parameters in ultrapure water

Quality parameters using the proposed CE–MS method with both hydrodynamic injection and electrokinetic injection were obtained and the figures are given in Table 3. The LODs based on a signal-to-noise ratio of 3:1 were lower than 3 mg l^{-1} when hydrodynamic injection (10 s) was used. Using electrokinetic injection (10 kV) the LODs decreased to 5–30 μg l^{-1} when the samples were injected during 10 s and to 1–10 μg l^{-1} if 20 s were used. These LODs are similar than those obtained using a quadrupol as analyser [28]. The molecular ions (for CQ, MQ and DF) and the radical molecular ion (for PQ) have been used for CE–MS quantitation purposes. Nevertheless, for DQ the relative abundance of the ions at m/z 184 ($[Cat]^{+\cdot}$) and 183 ($[Cat\text{-}H]^+$) was not constant so the signal of both ions (m/z 183+184) was monitored. Calibration curves based on the peak area ratio ($A_{compound}/A_{internal\ standard}$ or $A_{compound}/A_{labelled\ compound}$) for the five herbicides at concentrations between 2.5 and 40 mg l^{-1} (hydrodynamic injection, 10 s) and between 0.01 and 0.8 mg l^{-1} (electrokinetic injection, 10 s) and using EV and HV as internal standards were obtained showing acceptable linearities ($r>0.924$). Better results ($r>0.993$) were achieved by using labelled compounds as internal standards. Six replicate determinations of a standard solution of the five herbicides at ~15 mg l^{-1} (hydrodynamic injection) and ~0.3 mg l^{-1} (electrokinetic injection) were carried out under optimum conditions to determine run-to-run reproducibility and the relative standard deviations (RSDs) based on concentration ranged between 7 and 12% for hydrodynamic injection and between 11 and 17% for electrokinetic injection. Nevertheless, when labelled compounds were used as internal standard the run-to-run reproducibility improved obtaining RSDs between 2 and 5% and between 8 and 13% for hydrodynamic and electrokinetic injection, respectively. Moreover good accuracy was achieved and the best results were obtained when labelled compounds were used (bias lower than 8.5%).

Table 3
CE–MS quality parameters (ultrapure water)

Compound	LOD			Run-to-run ($n=6$)			
	Hydrodynamic injection, 10 s (μg l^{-1})	Electrokinetic injection		Hydrodynamic injection (10 s)		Electrokinetic injection (10 s)	
		10 s (μg l^{-1})	20 s (μg l^{-1})	I.S. (EN, HV)[a] (% RSD)	Labelled I.S.[b] (% RSD)	I.S. (EN, HV)[a] (% RSD)	Labelled I.S.[b] (% RSD)
PQ	2000	30	1	7	4	17	10
DQ	3000	30	10	8	5	11	8
CQ	1000	8	1	12	2	17	8
MQ	1000	8	1	12	3	14	9
DF	500	8	1	10	2	16	13

[a] EV for PQ and DQ; HV for CQ, MQ and DF.
[b] PQ-d_8 for PQ; DQ-d_4 for DQ; CQ-d_4 for CQ, MQ and DF.

For the CE–MS–MS study, the LODs based on a signal-to-noise ratio of 3:1 were obtained. With hydrodynamic injection, the LODs were in the range 1–3 mg l^{-1} and with electrokinetic injection (20 s, 10 kV) were in the range 1–10 μg l^{-1}. When CE–MS–MS was used, the noise decreased three times with respect the CE–MS noise, and the signal of the product ion also decreased in the same proportion. For this reason, the detection did not increase and the LODs obtained using CE–MS–MS were similar than those obtained with CE–MS. Then, CE–MS is proposed for quantitation and CE–MS–MS is only proposed for confirmation purposes.

3.5. Application

A highly contaminated irrigation water was analysed using the CE–MS method with hydrodynamic injection. Fig. 2 shows the electropherogram obtained for this sample. Two quaternary ammonium herbicides, PQ and DQ, were identified and quantified using CE–MS, and CE–MS–MS was used for confirmation.

The analysis of the sample was performed by standard addition ($n=3$) and using the labelled compounds PQ-d_8 and DQ-d_4 as internal standards. The results of the analysis were 12.5±0.5 mg l^{-1} for PQ and 6.2±1.2 mg l^{-1} for DQ. Moreover, this sample was also analysed by LC–MS using a chromatographic method previously developed [19] and the results obtained were in agreement (PQ: 11.3±0.8 mg l^{-1}, DQ: 7.6±0.3 mg l^{-1}) with those of CE–MS. Lower analysis times and similar precision were obtained for both techniques although

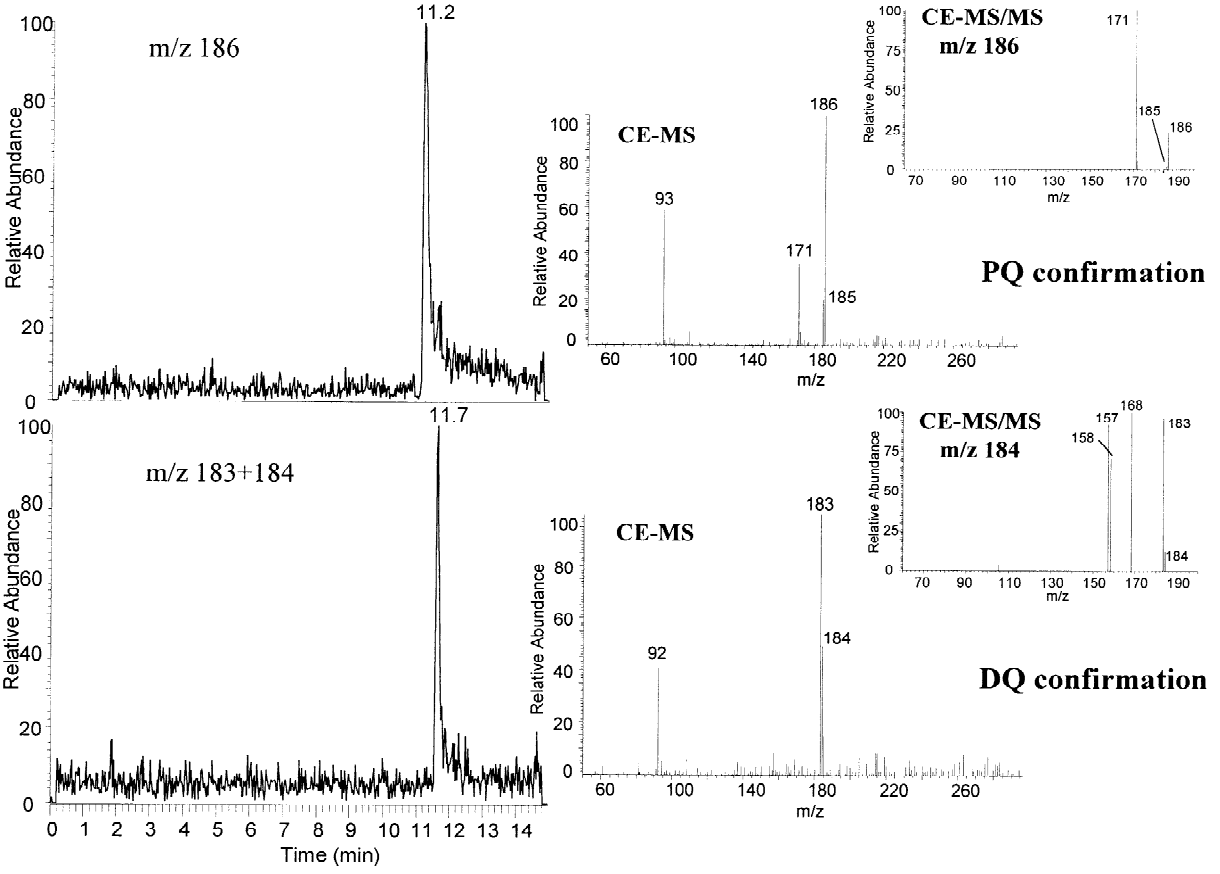


Fig. 2. Electropherogram of a contaminated irrigation water sample and CE–MS and CE–MS–MS spectra of the identified compounds. Electrophoretic conditions as in Fig. 1.

LC–MS provided lower detection limits (0.1–7 μg l^{-1}) [33].

In order to study the applicability of the method for the analysis of these compounds in drinking water at low levels, an spiked mineral water was also analysed by CE–MS using electrokinetic injection (20 s, 10 kV). The water sample was spiked with quats at ~20 μg l^{-1} (EPA level for DQ) and the electropherogram obtained is given in Fig. 3. LODs in mineral water in both CE–MS and CE–MS–MS were calculated obtaining values slightly higher than the figures for ultrapure water (10 μg l^{-1} for DQ and around 5 μg l^{-1} for the other quats). Both MS methods gave similar LODs probably due to the decrease of the signal and the noise in MS–MS as happened with standards and to the fact that drinking

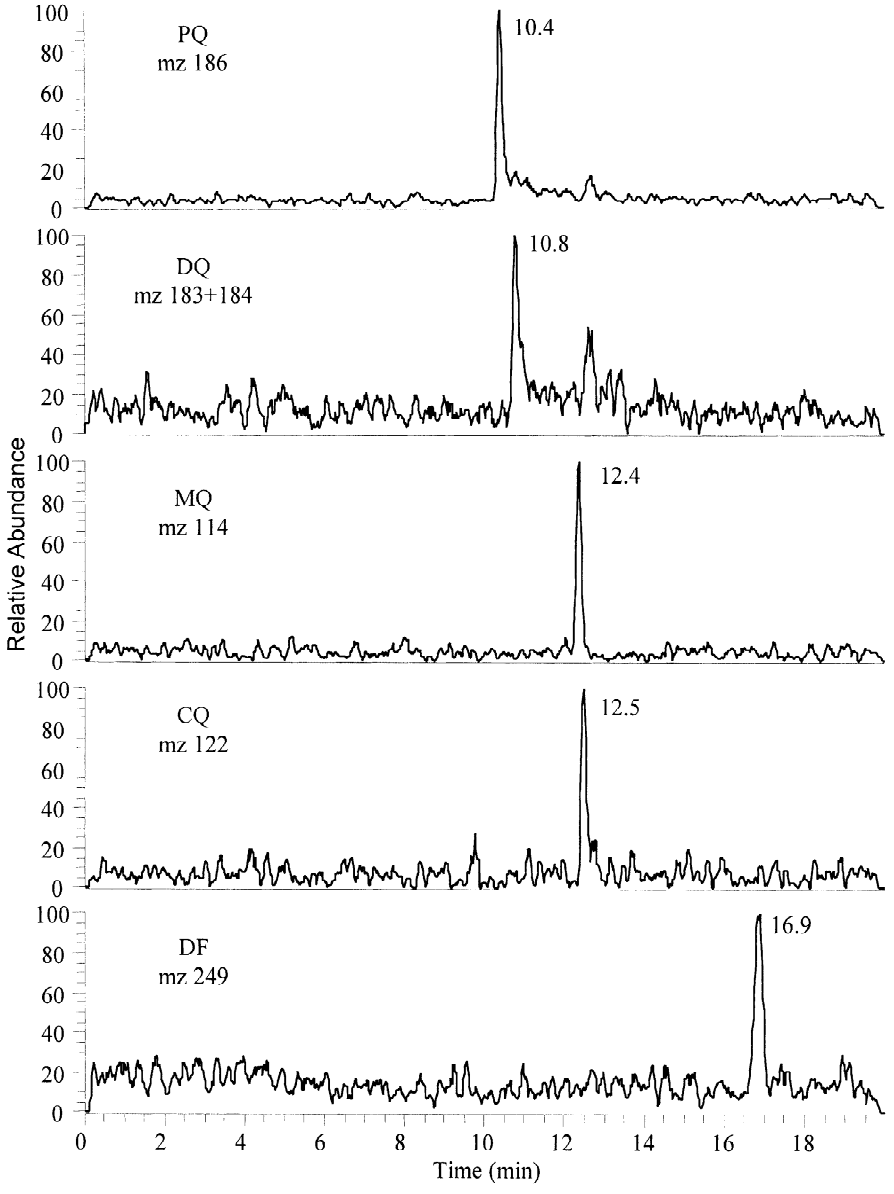


Fig. 3. Electropherogram of an spiked mineral water (~20 μg l^{-1}). Electrokinetic injection (20 s, 10 kV). Other conditions as in Fig. 1.

water is a relatively clean sample with a low chemical noise. LODs are below the maximum admissible level established by the US EPA for DQ showing that the method can be used for the analysis of quats in drinking water. Three analyses were performed by standard addition ($n=3$) of a mineral water spiked at 20 μg l^{-1} obtaining good accuracy and precision (from 2 to 10%).

4. Conclusions

A CE–MS (ion-trap) method using electrospray as ionisation source and a coaxial coupling device has been developed. Good reproducibilities and good electrophoretic separation were achieved avoiding the ionic suppression of the pairs PQ/DQ and CQ/MQ. CE–MS is proposed for quantitation and CE–MS–MS only for confirmation purposes because detection limits using this last technique did not improve in respect to CE–MS. For mineral water, LODs between 5 and 10 μg l^{-1} were achieved allowing the analysis of DQ in drinking water samples at the level established by the US Environmental Protection Agency. The results obtained showed that the method is a promising one for the identification and determination of these compounds when electrokinetic injection and labelled standards are used.

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