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Determination of quaternary ammonium pesticides by liquid chromatography–electrospray tandem mass spectrometry

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Abstract

A method for the direct determination of paraquat, diquat, chlormequat and difenzoquat in water samples, using an on-line solid-phase extraction–liquid chromatography–tandem mass spectrometry system was developed. No sample preparation was required and the detection limits were below the European Union maximum residue levels. The chromatographic separation was performed using an XTera MS C_8 column. The concentration of the ion pair reagent, the pH and the gradient elution were optimized to give high recoveries and good chromatographic resolution between quats. The detection was carried out using an ion trap as mass analyzer. Parameters such as the magnitude and duration of the resonant excitation voltage and the magnitude of the trapping RF voltage for full scan tandem mass spectrometry (MS–MS) experiments were studied to establish the optimal experimental conditions. Moreover, the accurate optimization of these parameters allowed MS–MS experiments of low mass ions, below m/z 200, providing unambiguous peak identification. Finally, the reproducibility of the proposed method was shown by good run-to-run and day-to-day precision values and its applicability to the determination of quats in drinking water was evaluated using spiked samples.

Keywords: Water analysis; Pesticides; Quaternary ammonium compounds

1. Introduction

The increasing concern about environmental pollution and drinking water and food contamination produced by the widespread use of pesticides has led to the establishment of strict regulations and has driven the efforts to develop highly sensitive analytical methods [1]. Quaternary ammonium compounds constitute an important group of pesticides. Paraquat (PQ) and diquat (DQ) are non-selective contact herbicides used for the control of weeds and grasses in fruit orchards and plantation crops, as defoliants for cotton and hops, as desiccants and for control of aquatic weeds. Difenzoquat (DF) is a selective herbicide used for the post-emergence control of wild oats in cereal crops. Chlormequat (CQ) is a plant growth regulator used to prevent lodging and increase yielding in cereals and ornamental plants and to promote flower formation and improve fruit setting in fruits and vegetables. Mepiquat (MQ) is also a plant growth regulator used on cotton to reduce vegetative growth and to inhibit sprouting [2]. For drinking waters the US Environmental Protection Agency has established a maximum contaminant level of 20 $\mu g\ l^{-1}$ for DQ and a goal of 3 $\mu g\ l^{-1}$ for PQ [3,4]. The European Union has not regulated the levels of these compounds in drinking water and continues to apply the values of 0.1 $\mu g\ l^{-1}$ for individual pesticides and 0.5 $\mu g\ l^{-1}$

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for total pesticides [5]. To achieve the legislated values, a trace enrichment procedure has to be employed. Solid-phase extraction (SPE) methods using silica [6,7] and C_8 [8,9] and C_{18} [10,11] bonded phases and graphitic carbon [12,13] have been reported.

The analysis of these compounds is difficult and although some methods have been published, liquid chromatography (LC) with UV detection [8,14] is the most frequently used. Nevertheless, the US Food and Drugs Administration recommends the use of mass spectrometry using at least three ions to confirm the presence of the compound [15]. Mass spectrometry methods have been reported for quats using different techniques, such as fast atom bombardment MS [16], CE–MS [17,18] and LC–MS [19,20]. When coupling liquid chromatography to mass spectrometry, the electrospray is recognized as the ionization technique of choice for the determination of quats. Since electrospray usually generates a single pseudomolecular ion there is potential for ambiguity in peak identification due to isobaric chemical interferences. For this reason, selected-reaction monitoring (SRM) performed on triple quadrupole instruments [21–25] has become the preferred quantitative tool. Nevertheless, ion traps can perform full-scan tandem mass spectrometry (MS–MS) experiments without compromising the sensitivity when compared with SRM experiments. Moreover, the ion or ions to be used for quantitation may be selected either before or after acquisition, since a full product spectrum is obtained and they can be changed without the need of further analysis. In addition, the full-scan product spectra obtained can be used to rule out false positives [26,27]. The objective of this work was to develop an on-line SPE–LC–MS–MS method sensitive and robust enough to comply with the maximum residue levels legislated that allows an unambiguous confirmation of the presence of the compounds in the sample and is potentially capable of automation for routine analysis.

2. Experimental

2.1. Chemicals

The reagents were obtained from the following sources: DQ, DF, MQ and CQ were all purchased from Chemservice (West Chester, PA, USA), PQ from Riedel–de Haën (Seelze, Germany). Hepthyl viologen (HV) (TCI, Tokyo, Japan) and ethyl viologen (EV) (Aldrich, Milwaukee, WI, USA) were used as internal standards. Acetonitrile and methanol HPLC-gradient grade, sodium hydroxide, formic acid and ammonia solution 25% (analytical grade) were purchased from Merck (Darmstadt, Germany). Water was purified using an Elix 3 coupled to a Milli-Q system (Millipore, Bedford, MA, USA). Heptafluorobutyric acid (HFBA) was obtained from Sigma (Poole, UK). The solid-phase extraction cartridges (10 mm I.D.×2 mm) obtained from Spark Holland (Emmen, The Netherlands) were: Hysphere-C_8 EC (silica based octyl phase, sperical shape, end-capped, 8 μm), PLRP-S (polymeric phase, 15–25 μm), Hysphere-Resin GP (polydivinylbenzene, spherical shape, 5–15 μm), Hysphere-Resin SH (modified polystyrene–divinylbenzene, irregular shape, 15–25 μm) and Hysphere-C_{18} HD EC (silica based, high loading of octadecyl chains, spherical shape, end-capped, 8 μm).

Stock standard solutions of individual quats, 1 mg ml^{-1}, were prepared in water and the working solutions were obtained by dilution with mobile phase. All solutions were stored in plastic vials to prevent adsorption and were passed through a 0.45 μm nylon filter before use.

2.2. Chromatography

Chromatographic separation was performed using a Waters 2690 separation module (Milford, MA, USA) equipped with a quaternary solvent delivery system, autosampler and column heater. The column used was a XTerra MS C_8 (100×2.1 mm, 3 μm, Waters). Post-column addition was carried out using an LKB–HPLC Pump 2248 from Pharmacia (Bromma, Sweden) and a Valco stainless-steel tee.

Gradient elution was used for separation of quats; solvent A was an HFBA aqueous solution (20 m*M*, pH 2.0) and solvent B was acetonitrile (20 m*M* HFBA). The elution program comprised a linear gradient from 0 to 5% of solvent B in 5 min and a stepwise to 40% of solvent B. For the on-line preconcentration the elution program was slightly modified, increasing the initial content of organic modifier, and comprised a linear gradient from 7 to

15% B and a stepwise to 60% B. The flow rate was 150 μl min^{-1} and the column temperature was maintained at 45°C. Post-column addition of methanol was carried out at a flow rate of 300 μl min^{-1}. A divert valve was placed before the mass spectrometer inlet and the flow was diverted to waste during the first 2 min.

2.3. Mass spectrometry

Mass spectrometry was carried out using an LCQ (Finnigan, San Jose, CA, USA) equipped with an electrospray ionization (ESI) source and operated using the Excalibur 1.0 SR1 software. A metallic needle was used for the sample inlet and an orthogonal sampling adapter (OSA) was placed in the heated capillary. Working conditions were the following: sheath gas flow rate 1.35 l min^{-1}, auxiliary gas flow rate 0.6 l min^{-1}, capillary temperature 275°C, capillary voltage 13.0 V, and spray voltage 3.0 kV. Data acquisition was performed in the full scan mode from m/z 100 to 370 for MS and from 50 to 300 for product ions in MS–MS with an injection time of 200 ms and 2 μscans. The Activation Q (AQ) was set between 0.32 and 0.34, the activation amplitude (AA) from 1.4 to 2.0 V and the activation time (AT) between 30 and 35 ms. Optimization of the ESI parameters was carried out using a standard solution of paraquat (10 μg ml^{-1}) introduced by infusion at a flow rate of 10 μl min^{-1}.

2.4. Sample treatment and on-line trace enrichment

On-line trace enrichment was carried out with SPE cartridges, using a HP-1050 quaternary pump (Hewlett-Packard, Palo Alto, CA, USA), a Rheodyne (Cotati, CA, USA) six-port switching valve model 7000 and a cartridge clamp (Spark Holland). A XTerra MS C_8 (30×2.1 mm, 3 μm, Waters) precolumn was added to the analytical column. The clamp was placed in the sample-loop position of the switching valve. The cartridges were conditioned with 10 ml of methanol, 10 ml of water and 10 ml of 20 m*M* HFBA adjusted at pH 7 with ammonium hydroxide. Water samples were then passed through the holder at a flow rate of 2.5 ml min^{-1}. Analytes were desorbed in the backflush mode through the analytical column at the chromatographic conditions previously described. The internal standard (EV) was injected (20 μl, 200 ng ml^{-1}) using the autosampler at the beginning of the chromatographic gradient. The switching valve was returned to the LOAD position within 30 s to avoid band broadening.

Drinking water samples were passed through a 0.45 μm nylon filter. A volume of HFBA was added to the sample to obtain a 20 m*M* solution and the pH was adjusted to 7 using ammonium hydroxide.

3. Results and discussion

3.1. Chromatographic separation

The previously published ion-pair LC–MS method [20] used a Kromasil C_8 column and an aqueous HFBA (15 m*M*, pH 3.3) as mobile phase, a gradient with acetonitrile (from 0.5 to 40% in 10 min, an isocratic period of 2 min and a stepwise to 60%). Although this system provided acceptable results, peak tailing occurred. Therefore, an alternative stationary phase was investigated in an attempt to improve peak shapes by reducing peak tailing. This was desirable in order to compensate for the ionization suppression effect that influences sensitivity and quantitative results. A C_8 XTerra (100×21 mm, 3 μm) was selected due to its extended pH working range (1–13) and its reduced surface silanol concentration.

When the original conditions were employed for the LC–MS coupling using the XTerra column, a very low retention was observed and peak tailing still remained. On silica-based reversed-phase columns, broad and tailing peaks have been attributed to the residual–SiOH groups. At low pH, peak shape of basic compounds is improved by suppressing ionization of silanols. In our case, the mobile phase was adjusted to pH 2 resulting in narrower and symmetrical peaks. Nevertheless, at these conditions very low retention times and coelution between PQ, DQ and MQ was obtained. As the problems with separation were presented only in the first part of the chromatogram, studies were carried out without internal standards and DF. Thus, a mobile phase without acetonitrile was used and the concentration of the ion-pair reagent was optimized to control the retention. The effect of the HFBA concentration from 10 m*M* to 20 m*M* is shown in Fig. 1. As can be

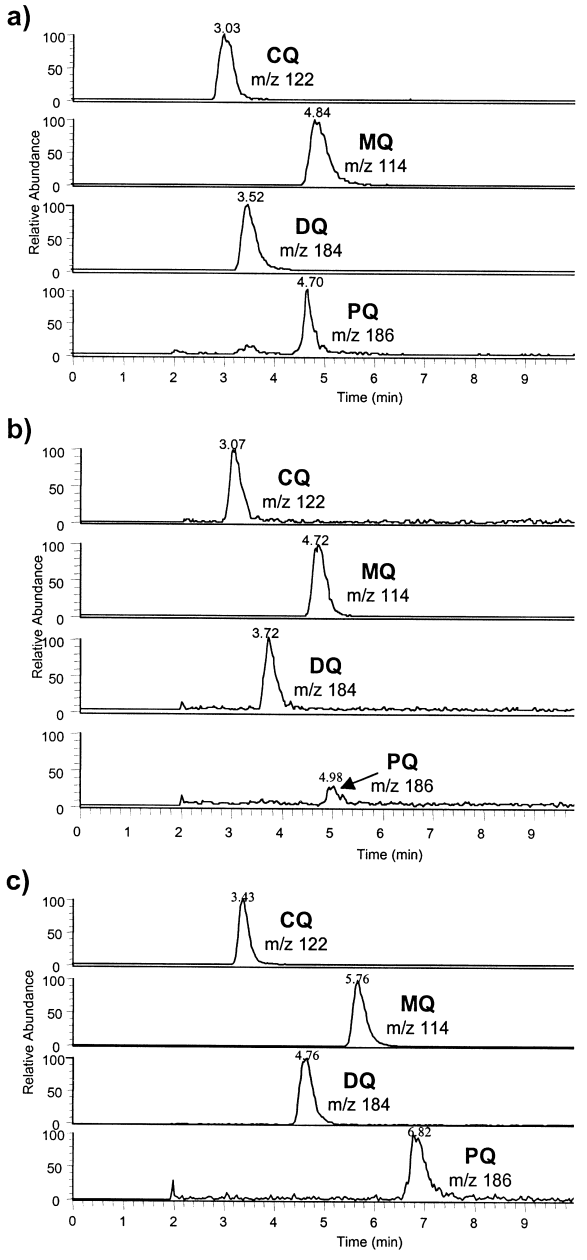


Fig. 1. Effect of ion-pair reagent concentration. (a) 10 m*M*, (b) 15 m*M*, (c) 20 m*M*.

observed, retention increased with increasing amounts of HFBA and a separation to the base line between CQ, DQ, PQ and MQ was obtained with 20 m*M* concentration. This value was used for the remainder of the study. At these conditions DF eluted at retention times higher than 30 min. To reduce analysis time, acetonitrile was added as organic modifier. Different conditions were tested and a good separation with similar analysis times were obtained using a linear gradient from 0 to 5% acetonitrile (20 m*M* HFBA) in 5 min and a subsequent increase of the acetonitrile content to 40%. When coupling LC to MS using this gradient, a very high spray current was obtained. In order to decrease this current, reduce the surface tension and improve MS response, the addition of an organic solvent was needed. A white film deposition on the ESI source was obtained when acetonitrile was used. To reduce this contamination methanol is recommended.

3.2. Mass spectrometry

The full scan MS spectra obtained by infusion in the conditions previously established, gave the molecular ion $[Cat]^+$ at m/z 122, 114 and 249 as the most abundant peak for CQ, MQ and DF respectively. For PQ and DQ, the base peak was the radical cation $[Cat]^{+\cdot}$ at m/z 186 and 184 respectively. These ions were used as precursors in MS–MS experiments. As a first step, the effect of the isolation width on precursor ion intensities was studied in order to obtain the maximum trapping efficiency without interferences of isotopic species or matrix components. It was found that for PQ, DQ, CQ, and MQ an isolation width of 1.5 could be applied while for DF an isolation width of 1.2 had to be used as higher values caused the $[^{13}C]$-Cat^+ ion to be trapped in addition to the $[^{12}C]$-Cat^+.

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. This CID energy is controlled by the magnitude (AA) and the duration (AT) of the resonant excitation voltage and the magnitude of the trapping radiofrequency (RF) voltage (AQ) [28,29]. To determine the stability range of both the precursor and product ions, the variation of ion intensities as a function of AQ was studied. For precursor ions the amplitude of the resonance excitation voltage (AA) was zero and for product ions values between 1.50 and 1.75 V (30–35%) depending on the compound were used. AT was maintained

at 30 ms and AQ was changed from 0 to 1.0. The curves obtained are given in Fig. 2a. Similar behavior was observed for all the quats: a wide stability range was obtained for the precursor ions while product ions were only stable in a narrow range which is dependent on the m/z of the product ion being narrower for small ions. Optimal values for AQ were those at which maximum intensity for product ions were obtained and are given in Table 1.

As mentioned above, increasing the resonance excitation voltage (AA) or the duration of this voltage (AT) the yield of product ions relative to the unfragmented precursor ion is enhanced. The effect of the variation of AA on both precursor and parent ions was studied. For each compound AQ was set at the value previously selected and AT at 30 ms. As can be observed in Fig. 2b, for PQ, DQ, CQ and MQ an AA>1.0 V (20%) was needed to obtain product ions but the fragmentation of DF required a higher value (1.5 V, 30%). The selected values for AA are given in Table 1. The effect of the AT in the precursor ion fragmentation was investigated by monitoring the variation of ion intensities at different AT values; AQ and AA were set at the previously selected values (Table 1). In general, optimal values for activation times were between 30 and 35 ms (Table 1).

The spectra generated under MS–MS conditions are given in Table 2. In general, the fragmentation of DQ, PQ and MQ that produced as base peaks the ions $[Cat–CNH]^{+\cdot}$, $[Cat–CH_3]^+$ and $[Cat–CH_3]^{+\cdot}$, respectively, agreed with the results obtained by other authors [22,23]. Nevertheless some differences were observed for CQ and DF. For instance, for CQ besides the main fragments at m/z 58 and 59, the ion trap instrument gave the ion at m/z 86 produced from the neutral loss of HCl and the ion at m/z 94 assigned to the $[Cat–C_2H_4]^+$. These ions were also observed by Evans et al. [30] with an ion trap, but did not appeared with triple quadrupole instruments [21,23,25] which in contrast yielded the $[Cat–N(CH_3)_3]^+$ at m/z 63. The probable mechanism explaining the ion at m/z 94 is that an ion/molecule reaction between the ion at m/z 59 and the HCl present in the ion trap occurred. In a similar way, the product ion at m/z 96 was obtained when the $[^{37}Cl]$-Cat^+ at m/z 124 was used as precursor ion. For DF the most important difference was the presence of the ion at m/z 208 that was not observed by the triple quadrupole instrument [23]. This m/z can be tentatively assigned to the ion $[Cat\text{-}(NCH_3)_2+2H+CH_3]^{+\cdot}$ obtained by the reaction between a $\cdot CH_3$ produced in the generation of m/z 234 and the ion at m/z 193. The presence of these ions demonstrates that reactions with neutral molecules and radicals occurred easily within the trap.

3.3. Online trace enrichment

To decrease detection limits a preconcentration procedure was employed. Trace enrichment was performed by percolating 10 ml of a 250 ng l^{-1} quats solution and eluting with the mobile phase previously optimized. At these conditions a dramatic peak broadening was observed. This effect was produced during the transfer from the cartridge to the analytical column and was due to the differences in the particle size between them. To avoid this, the time that the switching valve has to remain in the inject position was optimized. It was found that time values lower than 30s were needed to avoid band broadening. As an example in Fig. 3 the chromatograms at different switching valve times are shown. However, using 30 s as switching valve time, the recoveries were very low (from 20 to 50%). To improve the recoveries the initial content of organic modifier was increased. Nevertheless, at these conditions the separation between the PQ, DQ and MQ was degraded. To solve this problem a XTerra MS C_8 precolumn (30×2.1 mm, 3 μm) was used. The best recoveries were obtained with an acetonitrile gradient from 7 to 15% in 5 min and an increase to 60%. Different packings that were commercially available for automatic preconcentration were evaluated. To calculate the recoveries the peak areas were compared with those obtained by direct injection of the standard solution and the results are shown in Table 3. C_8 and C_{18} reversed-phases gave very low recoveries for CQ, MQ and DQ, which was not recovered using the C_{18} sorbent. Polymeric phases gave better recoveries for most of the quats and the best results were obtained with the polydivinylbenzene (Hysphere-Resin GP). So this sorbent was selected for the remainder of the study, although with this sorbent the recovery for the DF was relatively low.

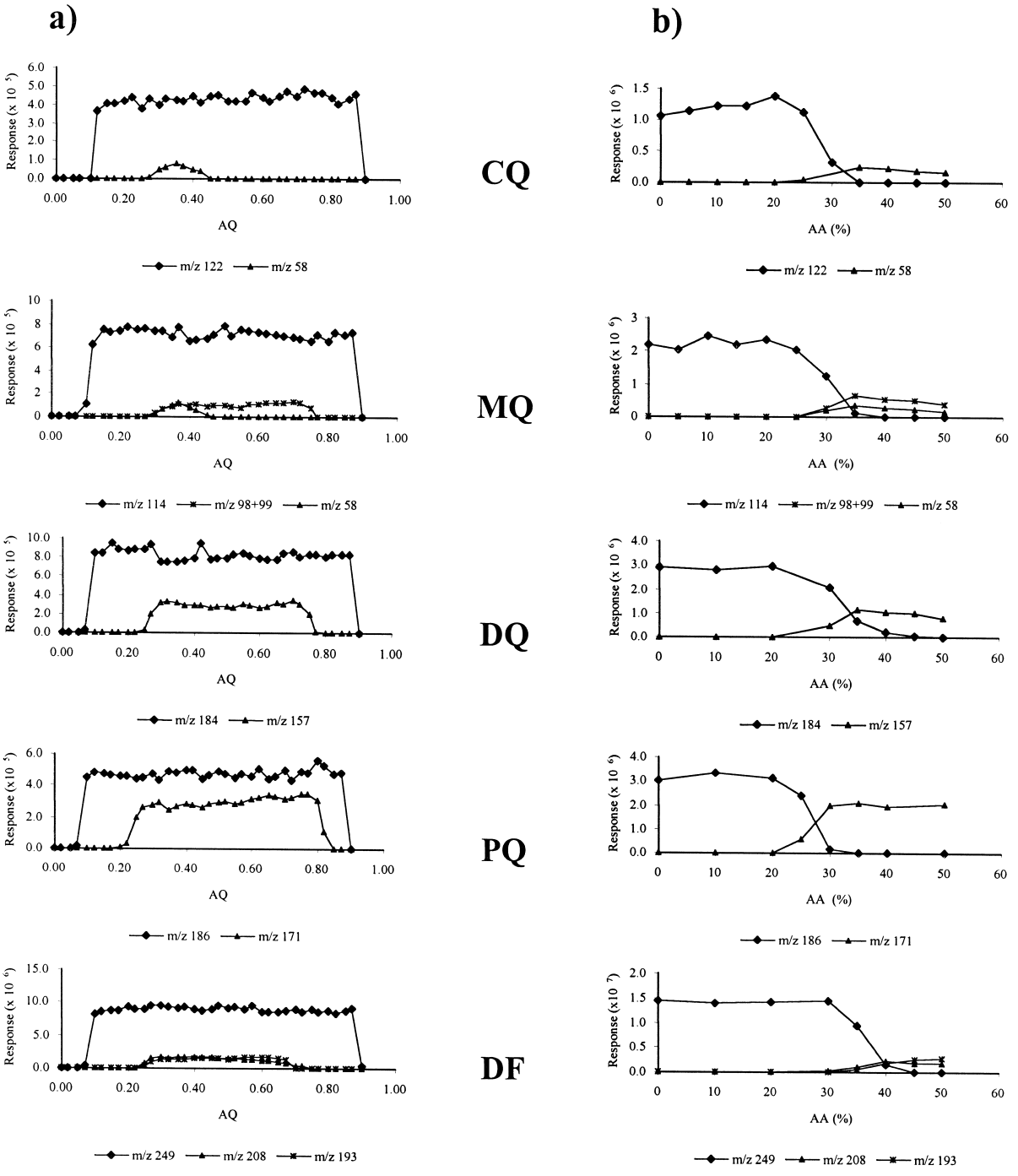


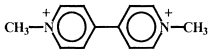
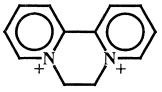
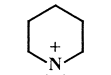
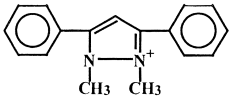
Fig. 2. Variation of precursor and product ions as a function of (a) trapping RF voltage (AQ) and (b) resonance excitation voltage (AA).

Table 1
Selected values for MS–MS parameters

Parameter	CQ	MQ	DQ	PQ	DF
Precursor m/z	122.3	114.3	184.2	186.3	249.4
Isolation width (m/z)	1.5	1.5	1.5	1.5	1.2
Activation Q	0.35	0.37	0.32	0.32	0.4
Activation amplitude (%/V)	32/1.6	35/1.75	36/1.8	28/1.4	40/2
Activation time (ms)	32	32	30	35	35

In order to obtain the breakthrough volume, volumes from 10 to 75 ml of spiked Milli-Q water were percolated through the cartridges at 2.5 ml min^{-1}. The concentration levels of the spiked samples were adjusted in order to preconcentrate a constant amount (25 ng) of each compound. Recoveries were calculated by dividing peak areas obtained when percolating a given volume by those obtained for the smallest volume at which breakthrough did not occur. For PQ, DQ, CQ and MQ good recoveries were obtained up to 30 ml, while DF presented a significant decrease at this volume. Fig. 4 shows the breakthrough curves obtained. Although it is generally assumed that it is better to work with

Table 2
Mass spectra data of quaternary ammonium herbicides in LC–MS–MS

	Precursor ion	m/z	Product ion	m/z
PARAQUAT (PQ)				
	$[Cat]^{+\cdot}$	186	$[Cat-CH_3]^+$	171
DIQUAT (DQ)				
	$[Cat]^{+\cdot}$	184	$[Cat-NH_2]^+$ $[Cat-CNH]^{+\cdot}$	168 157
MEPIQUAT (MQ)				
	$[Cat]^+$	114	$[Cat-CH_3]^{+\cdot}$ $[Cat-CH_3-H]^+$ $[Cat-C_4H_8]^+$	99 98 58
CHLORMEQUAT (CQ) + $ClCH_2CH_2N(CH_3)_3$	$[Cat]^+$	122	$[Cat-C_2H_4]^+$ $[Cat-HCl]^+$ $[Cat-ClC_2H_5]^{+\cdot}$ $[Cat-ClC_2H_4]^+$	94 86 59 58
DIFENZOQUAT (DF)			$[Cat-CH_3]^{+\cdot}$	234
	$[Cat]^+$	249	$[Cat-(NCH_3)_2+2H+CH_3]^{+\cdot}$ $[Cat-(NCH_3)_2+2H]^+$ $[Cat-C_6H_5-CNCH_3]^{+\cdot}$ $[Cat-C_6H_5-C_2HNCH_3]^+$	208 193 131 118

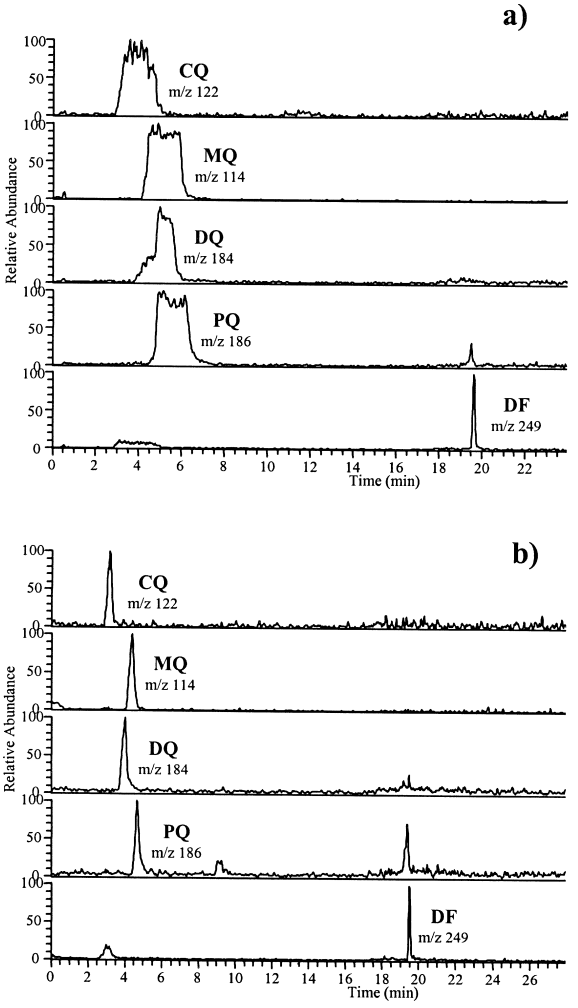


Fig. 3. Effect on of switching valve time on peak shapes, (a) 2 min, (b) 30 s, Hysphere-Resin GP cartridges. Working conditions as described in Section 3.3.

Table 3
Recoveries of quats with different sorbent after preconcentration

Sorbent	Recovery (%)				
	CQ	MQ	DQ	PQ	DF
C_8 EC	7.0	23.9	29.3	96.1	90.1
PLRP-S	17.5	51.6	47.6	98.3	30.7
Resin GP	78.9	94.8	102.9	90.0	44.4
Resin SH	65.4	76.5	58.4	88.4	12.5
HySphere-1	46.8	53.1	44.2	65.8	1.2
C_{18} HD EC	8.0	22.1	N.R.[a]	60.5	43.5

[a] N.R.: not recovered.

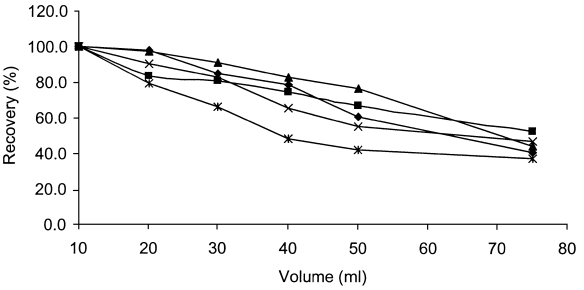


Fig. 4. Breakthrough curves of quats (25 ng) obtained with Hysphere-Resin GP cartridges. Working conditions as described in Section 3.3.– ♦ – CQ, – ■ – MQ, –▲– DQ, – × – PQ and –*– DF.

high recoveries, a sample volume of 30 ml was chosen to obtain an amount of DF as high as possible.

3.4. Quality parameters

To evaluate the analytical performance of the proposed method, figures of merit were determined. Detection limits (LODs), based on a signal-to-noise ratio of 3:1 were estimated in Milli-Q water spiked at low levels. These limits are given in Table 4. LODs for full scan MS–MS were found to be about ten-fold better than with full-scan MS. The improvement obtained was produced by the filtering effect of removing all background ions. Moreover, on-line preconcentration allowed detection limits from 0.01 to 0.04 $\mu g\ l^{-1}$. In global, SPE–LC–MS–MS permitted an increase in sensitivity of ca. 200 times. These detection limits are lower than the legislated levels for drinking water.

Day-to-day and run-to-run precisions were established by preconcentrating 30 ml of Milli-Q water spiked at 250 ng l^{-1} and quantifying using calibration curves constructed by preconcentrating Milli-Q water standard solutions between 50 and 500 ng l^{-1} and EV as internal standard. The run-to-run precision of the on-line extraction method was determined by six replicates carried out on the same day. Relative standard deviations (RSDs) between 8 and 11% were obtained. The day-to-day precision was determined with six replicates carried out on three different days. RSDs from 10 to 14% were

Table 4
Quality Parameters

	CQ	MQ	DQ	PQ	DF
LOD (μg l^{-1})					
LC–MS	10	5	15	25	1
LC–MS–MS	1	0.5	1	5	<0.1
On-line SPE–LC–MS–MS	0.02	0.01	0.03	0.03	0.04
Precision					
Run-to-run (%) (n=6)	8.5	8.9	9.4	7.9	11.2
Day-to-day (%) (n=6×3 days)	9.8	11.4	12.8	10.1	14.5

obtained. These results showed that good precisions can be attained with the method described above.

3.5. Application

In order to test the applicability of the method a still mineral water sample free of quats was spiked at the EC maximum residue level (100 ng l^{-1}). Fig. 5 gives single-ion chromatograms for each characteristic fragment and the full-scan MS–MS spectra of each compound obtained after the on-line preconcentration of the spiked water sample. As can be seen, on-line SPE–LC–MS–MS is a highly selective procedure for the analysis of quats in water, showing no interference from other compounds potentially present in the sample matrix. For the quantitative

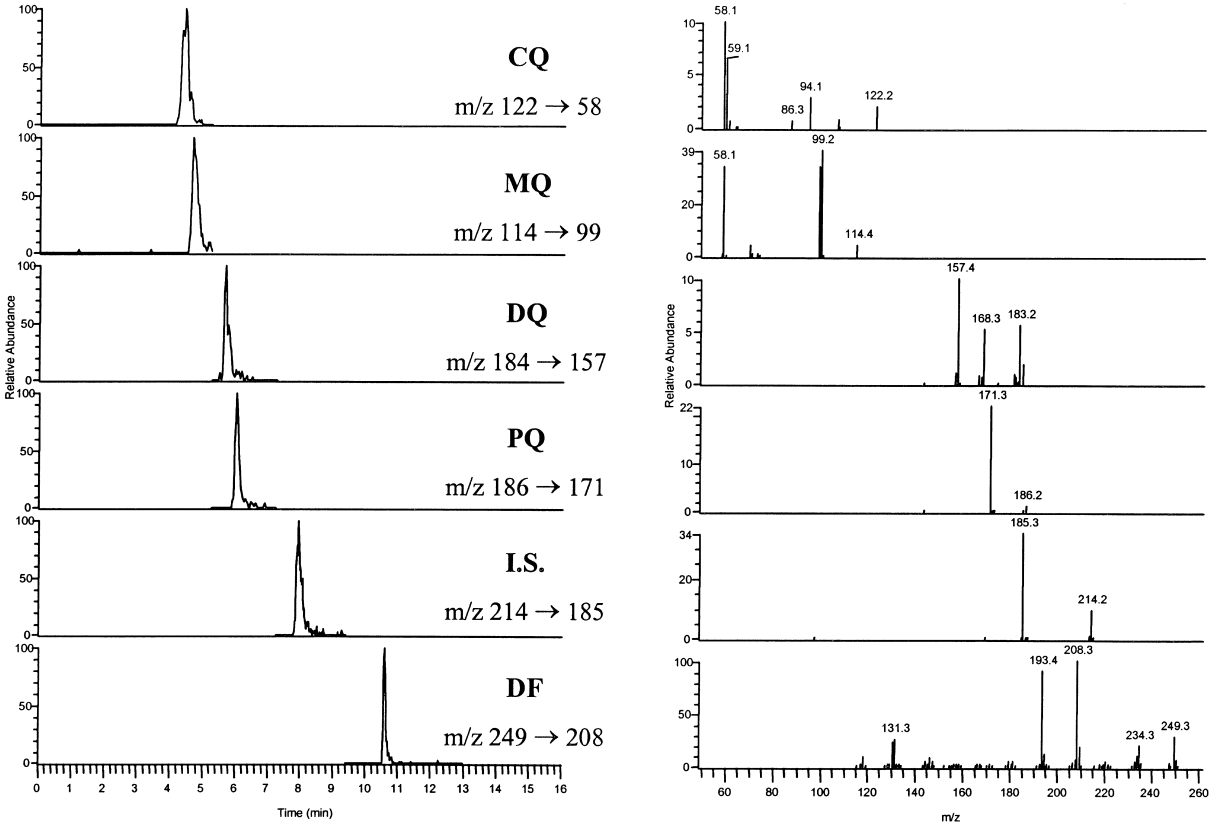


Fig. 5. On-line SPE–LC–MS–MS chromatogram obtained for a drinking water sample spiked at 100 ng l^{-1}. I.S.: ethyl viologen.

Table 5
Determination of quats in a spiked sample using on-line SPE–LC–MS–MS

	Expected value (ng l^{-1})	Standard addition		External calibration		Significance level (P-value)[b]
		Mean (ng l^{-1})[a]	RSD (%)	Mean (ng l^{-1})[a]	RSD (%)	
CQ	97.4	92.9	10.3	102.4	11.6	0.350
MQ	99.4	94.0	10.8	106.0	11.6	0.258
DQ	100.0	96.3	10.7	114.3	12.7	0.134
PQ	97.6	94.4	9.9	100.9	11.5	0.496
DF	105.7	98.2	13.3	94.0	14.2	0.727

[a] $n=3$.
[b] Significant differences between procedures for $P<0.05$ (at the 95% confidence level).

determination two methods, external calibration and standard addition, were used. Aliquots of 30 ml were treated as described in Section 2.4. For external calibration, curves were generated from Milli-Q water standard solutions from 50 to 400 ng l^{-1}, while standard addition was carried out by adding to the sample a standard solution at five concentration levels between 50 to 400 ng l^{-1}. Samples were analyzed in triplicate and EV was used as internal standard. The values obtained for the determination of quats in the spiked sample are shown in Table 5. The analytical significance of the mean values of the two quantification methods was statistically studied using the *t*-test. Variances were checked to be equivalent using the *F*-test. The significance values (P) obtained are given in Table 5. No significant differences were observed between the two methods for any compound. Sample matrix did not influence the quantitative analysis and accurate results were obtained using both quantification methods. As a consequence external calibration can be proposed for the quantitative analysis as an important time and work saving advantage.

4. Conclusions

The proposed method, involving an on-line SPE procedure using polydivinyl-benze cartridges coupled to an XTerra MS C_8 LC column and MS–MS detection allowed the analysis of quats at low concentration levels. The low detection limits (from 0.01 to 0.07 μg l^{-1}) and the precision (below 14%) obtained were good enough to ensure a reliable determination at the legislated drinking water levels. Moreover, tandem mass spectrometry provided a very specific fragmentation information resulting in confident identification of the compounds. Consequently, on-line SPE–LC–MS–MS using external calibration can be proposed for the routine analysis of quats in water samples with an unambiguous identification. Moreover, this method avoided tedious concentration steps that are time consuming and could easily be automated.

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