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On-line ion-pair solid-phase extraction–liquid chromatography–mass spectrometry for the analysis of quaternary ammonium herbicides

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Abstract

An ion-pair on-line solid-phase extraction procedure using C_8 extraction disks, suitable for liquid chromatography–mass spectrometry analysis is developed to determine quaternary ammonium herbicides (quats) in water samples. The separation of these compounds was performed using ion-pair chromatography with heptafluorobutyric acid (15 m*M*, pH 3.3) and acetonitrile gradient elution. Detection was carried out using a quadrupole mass spectrometer. Water sample volumes up to 50 ml can be preconcentrated with recoveries higher than 70%. Good precision and accuracy (day-to-day and run-to-run) were obtained and the detection limits ranged from 6 to 85 ng l^{-1}. The proposed on-line ion-pair solid-phase method enables compliance with European Community directives for drinking waters (100 ng l^{-1}).

Keywords: Water analysis; Extraction methods; Pesticides; Quaternary ammonium compounds

1. Introduction

Quaternary ammonium pesticides, known as ‘quats’, are widely used in agriculture in many countries. Paraquat (PQ), diquat (DQ) and difenzoquat (DF) are used mainly as herbicides, with chlormequat (CQ) and mepiquat (MQ) being used as growth regulators [2]. Due to their high levels of use, growing concern has recently been expressed at the presence of compounds of this kind in foods, water and soils. On the basis of their toxicity, the World Health Organization has classified PQ, DQ and DF as moderately hazardous and CQ and MQ as slightly hazardous [3]. Given the threat they pose to the environment, some of these compounds have been included on ‘priority’ lists and are currently regulated in a number of countries [4]. For drinking waters, the Office of Water of the US Environmental Protection Agency (EPA) has established a health advisory level of 20 μg l^{-1} for DQ and a maximum contamination level goal of 3 μg l^{-1} for PQ [5]. The European Community has not regulated the levels of these compounds in water and the values 100 ng l^{-1} for individual pesticides and 500 ng l^{-1} for total pesticides are applied [1]. To comply with these regulations, analytical methods for quats still need to be developed.

Since quats exist as charged cationic species, their analysis is difficult and although a great number of chromatographic [6,7], ion-selective [8,9] and spectrophotometric [10,11] methods have been developed, liquid chromatography (LC) [12,13] and capillary electrophoresis (CE) [12,14] with UV detection are the techniques most frequently used for the analysis of these compounds. Nevertheless, a

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major disadvantage of these methods is the lack of analytical specificity, which results in identification and quantification difficulties. The use of mass spectrometry as a detection technique allows these problems to be overcome; however, although some studies using LC [15–17] and CE [18,19] coupled to mass spectrometry have been published, these methods do not yet have limits of detection (LODs) as low as the European Community requirements (100 $ng\,l^{-1}$ for pesticides) in drinking waters. Consequently, a trace enrichment step before chromatographic analysis is required.

Due to its simplicity and economy in terms of time and solvents needed, solid-phase extraction (SPE) is the method selected here. Off-line SPE procedures involving different packing materials such as C_8 [20,21] and C_{18} [22,23] bonded phases, silica [24,25], porous graphitic carbon [26] and cation-exchangers [27] have been reported. The EPA method 549.1 [20] employs C_8 cartridges and sodium heptanesulfonate as ion-pair reagent. Nevertheless, in the analysis of low concentration levels of organic compounds, the recovery problems take on paramount importance. In order to minimize sample losses and the risk of contamination, sample handling needs to be reduced to a minimum. When using off-line SPE procedures, trace compounds are trapped in a sorbent and are recovered later by elution with a small volume of organic solvent, this volume has to be reduced and frequently, only a small fraction is introduced in the analytical system. Many of these inconveniences can be overcome by using on-line trace systems. Among their advantages are lower detection limits, no evaporation losses, no contamination and ease of automation [28]. For the analysis of quats, the use of on-line SPE using silica [29], gel filtration [30] and graphitic carbon [31] packings followed by LC with UV detection have been reported. Nevertheless, although generally the off-line SPE methods can easily be converted into on-line LC systems, most of these methods cannot be adapted for the on-line LC–MS analysis as they incorporate non-volatile buffers or ion-pair reagents.

Elsewhere [32], we described an ion-pair LC–MS method for the determination of quats in water samples with an off-line trace enrichment procedure using silica cartridges. Since the LODs achieved with this method were not low enough to comply with the European Community directive and it cannot be used as an on-line setup, in this paper we study the application of an ion-pair on-line solid-phase extraction procedure suitable for coupling to the LC–MS method previously published.

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). HPLC-gradient grade acetonitrile (ACN) and methanol, 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). Ethyl viologen (Aldrich, Milwaukee, WI, USA) was used as internal standard. Envi-8 DSK 47-mm solid-phase extraction disks were obtained from Supelco (Bellefonte, PA, USA).

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. Chromatographic conditions

Chromatographic separation was performed using a Waters 2690 separation module (Milford, MA, USA) equipped with a quaternary solvent delivery system and an autosampler. The column used was a Kromasil C_8 (200×2.1 mm, 5 μm; Tracer Analitica, Spain). Column temperature was regulated using a CH-30 column heater with a TC-50 temperature controller (Eppendorf, Madison, WI, USA). Acetonitrile post-column addition was carried out using a HP-1050 quaternary pump (Hewlett-Packard, Palo Alto, CA, USA) and a Valco stainless-steel tee.

Gradient elution was used for optimal separation of quats; solvent A was an aqueous HFBA solution

(15 m*M*, pH 3.3) and solvent B was acetonitrile. The elution program comprised a linear gradient from 12 to 40% of solvent B in 7 min and a stepwise elution from 40 to 60% of solvent B. The flow-rate was 200 $\mu l\,min^{-1}$. The temperature of the column was maintained at 50°C and the post-column acetonitrile addition was 400 $\mu l\,min^{-1}$.

2.3. Mass spectrometry conditions

Mass spectrometry was carried out using a VG Platform II (Fisons Instruments, VG Biotech, Altrincham, UK) quadrupole mass spectrometer equipped with a pneumatically-assisted electrospray (ESI) source.

Working conditions for mass spectrometer were the following: the source was heated to 160°C, the capillary potential was +3.5 kV and the extraction voltage 40 V, nebulizing N_2 flow-rate was 20 $l\,h^{-1}$ and drying N_2 flow-rate 400 $l\,h^{-1}$. Data acquisition was performed in selected-ion monitoring (SIM) mode with a dwell time of 100 ms and an inter-channel time of 1 ms. The $[Cat]^+$ ion for MQ, CQ and DF and the $[Cat\text{-}H]^+$ ion for PQ and DQ were the recorded ions. In order to optimize ESI parameters, a standard solution of paraquat (10 $\mu g\,ml^{-1}$) was introduced by flow injection analysis (FIA).

2.4. Sample treatment and on-line trace enrichment

Drinking water samples were passed through a 0.45-μm nylon filter. A volume of 200 μl of HFBA was added to 100 ml of sample to obtain a 15 m*M* solution and the pH was adjusted to 9 using 10% (w/v) sodium hydroxide.

The on-line trace enrichment was performed with an LKB-HPLC Pump 2248 from Pharmacia (Bromma, Sweden) and a Rheodyne (Cotati, CA, USA) six-port switching valve model 7000. ENVI-8 disks (4.6-mm diameter) in a stainless-steel disk holder and the cutting device were purchased from the Free University of Amsterdam, Netherlands [33]. Each conventional 47-mm I.D. disk provides ca. 40 minidisks and, therefore, various precolumns can be prepared.

The general scheme for the on-line preconcentration was as follows: the holder, containing 8 ENVI-8 extraction disks and two 0.45-μm nylon membrane disks (one on each side), was placed in the sample-loop position of the switching valve. The disks, conditioned with 10 ml of methanol, were then equilibrated with 10 ml of water and 10 ml of 15 m*M* HFBA adjusted at pH 9 with 10% sodium hydroxide. Water samples were then passed through the holder containing the extraction disks at a flow-rate of 2 $ml\,min^{-1}$. The valve was switched to the INJECT position and at the same time the chromatographic gradient was started, eluting quats in the backflush mode through the analytical column at the chromatographic conditions previously described. The internal standard was injected (20 μl, 650 $ng\,ml^{-1}$) using the autosampler at the beginning of the chromatographic gradient. The switching valve was returned to the LOAD position within 45 s to avoid band broadening.

3. Results and discussion

3.1. On-line trace enrichment

The off-line trace enrichment procedure previously reported [32], which used silica cartridges and 6 *M* HCl as eluting solution, was not compatible with the ion-pair LC–MS. To develop an on-line trace enrichment procedure, different sorbents [C_{18}, and PRP-1 precolumns; C_{18}, poly(styrene–divinylbenzene) (PS–DVB) and C_8 extraction disks], ion-pair reagents [sodium heptanesulfonate (SHS), HFBA, pentafluoropropionic acid (PFPA), trifluoroacetic acid (TFAA)] and working conditions (concentration, pH, switching valve time, initial acetonitrile percentage) were studied. Experiments were carried out by percolating 10 ml of a PQ and DQ solution (250 $ng\,ml^{-1}$) and eluting later with the mobile phase (HFBA–ACN). To calculate the recoveries, the peak areas were compared with those obtained by direct injection of the standard solutions.

Preliminary studies were performed using PRP-1 and C_{18} precolumns and it was found that the recoveries for PQ and DQ, used as target compounds were very low if an ion-pair reagent was not incorporated into the sample. For instance, for TFAA, PFPA and HFBA a high concentration (500 m*M*) was needed to obtain acceptable recoveries

and, in addition, the breakthrough volumes were relatively low (20 ml of water). Better recoveries were obtained when SHS 100 m*M* was added to the sample, but a considerable peak broadening and loss of resolution between PQ and DQ in the chromatogram was observed. To prevent adsorption in the precolumn, the EPA method uses cetyl trimethyl ammonium in the conditioning step, nevertheless in our case the use of this compound produced a dramatic decrease in the recoveries.

When trace enrichment was performed using disks, better recoveries were obtained. When PS–DVB was used a change in the retention times with a loss of resolution between PQ and DQ was observed, so other SPE disks were tested. The best results were obtained using C_{18} and C_8 disks and HFBA at low concentration levels of only 15 m*M*. As the analytical column was a C_8, the same bonded phase was chosen for the preconcentration.

The influence of pH on the preconcentration of the quats was studied and it was found that lower recoveries were obtained in acidic than in alkaline conditions, although at pH higher than 10 recoveries decreased due to the degradation of the DQ and the increase of the adsorption of PQ [34]. Furthermore, it was observed that when the pH of both the conditioning solution and the sample was adjusted with sodium hydroxide rather than ammonium hydroxide, recoveries presented a marked increase. This might have been due to the high adsorption of the sodium ion on the deprotonated silanol groups preventing an irreversible adsorption of the quats.

When using on-line trace enrichment, it is recommendable to use a precolumn with smaller internal diameter than the analytical column in order to avoid band broadening during the transfer from the precolumn to the analytical column [28]. Since the internal diameter of our preconcentration holder was greater than that of the analytical column, it was necessary to establish the minimum time that the switching valve had to remain in the inject position in order to allow the total elution of the compounds and prevent band broadening. It was found that 45 s was enough for the total desorption of the analytes without a significant broadening of the peak. Elution in the backflush mode was used in order to obtain narrower peaks. Also, to obtain a faster desorption, the initial acetonitrile content must be 12%.

The amount and volume of a sample solution that can be preconcentrated depends on the number of disks in the holder, the characteristics of the sorbent, the nature of the conditioning solvent and the flow-rate used during sampling. The final enrichment factor obtainable is determined by the breakthrough of the solute and the solubility of the analyte in the mobile phase used for the elution to the analytical column [35]. In order to determine the breakthrough volume spiked, HPLC-gradient grade water sample volumes from 10 to 75 ml were percolated through the disk holder at 2 ml min^{-1}, analytes were eluted at 200 μl min^{-1} and peak areas were measured. The concentration levels of the spiked samples were adjusted in order to preconcentrate a constant amount (25 ng) of each compound. The lowest volume tested was such that breakthrough did not occur for any solute. This was verified comparing the peak areas obtained from a direct injection of the same amount of the analytes. A decrease in the peak area indicated that breakthrough had occurred. Recoveries were calculated by dividing peak areas obtained when percolating a given volume by those obtained for the smallest volume. Sample volumes higher than 75 ml were not studied to reduce the analysis time.

For PQ, DQ and DF good recoveries were obtained up to 75 ml, while CQ and MQ presented a significant decrease at volumes higher than 25 ml. Fig. 1 shows the breakthrough curves obtained by percolating spiked HPLC-grade water. When target compounds are numerous, it is almost impossible to

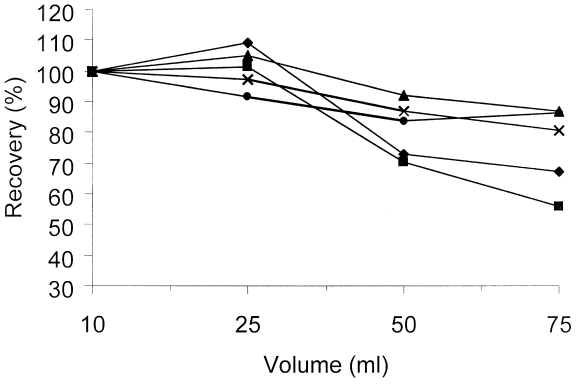

Fig. 1. Breakthrough curves of quats (25 ng), obtained with C_8 extraction disks. Experimental conditions are described in Section 2.4. —♦— CQ, —■— MQ, —▲— DQ, —×— PQ and —●— DF.

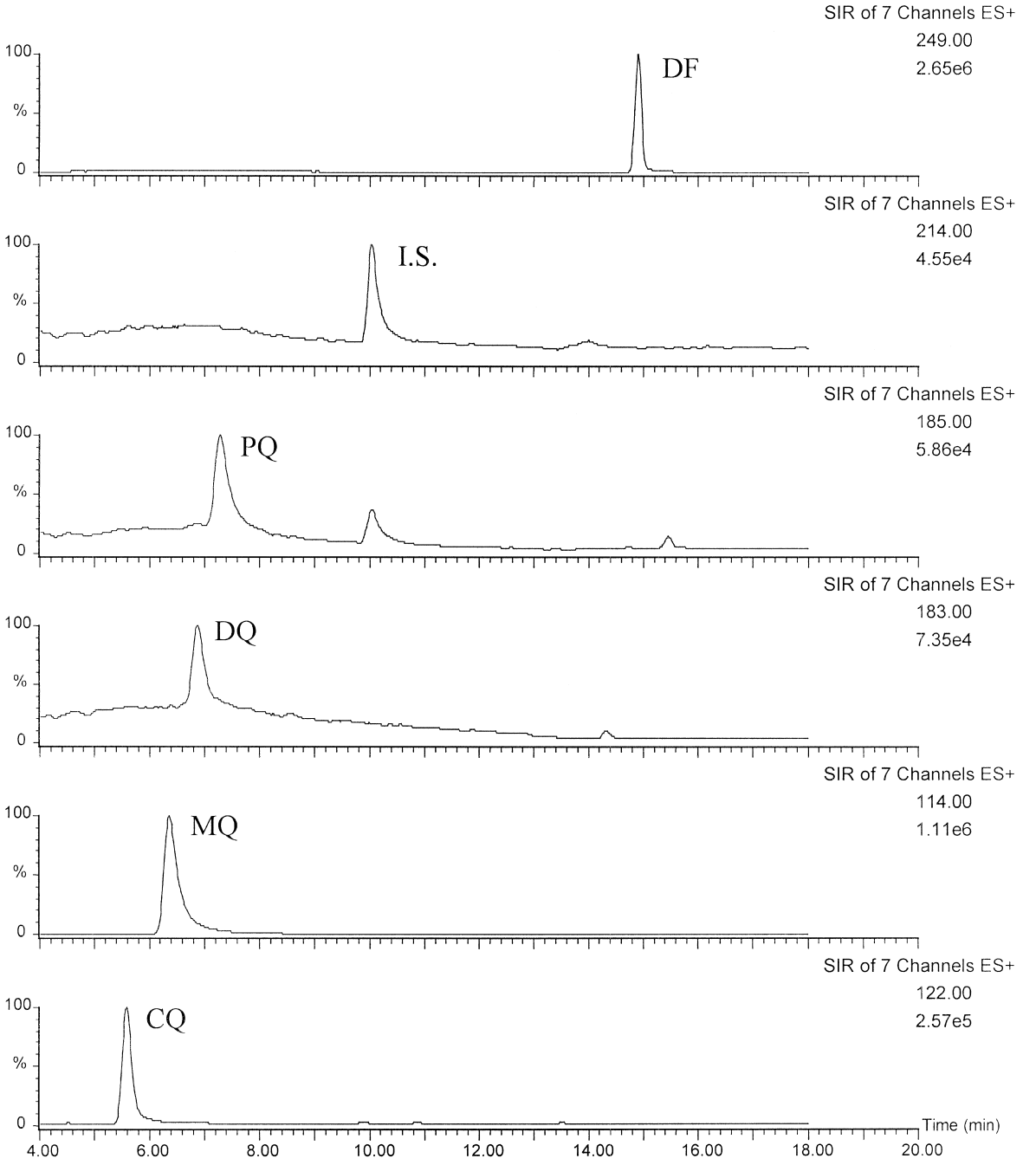

Fig. 2. On-line SPE–LC–ESI-MS chromatogram obtained after percolating 50 ml of a solution of quats in water at a concentration level of 500 ng l^{-1}. MS: acquisition data in SIM mode. Experimental conditions are described in Section 2.

Table 1
Detection limits of quats using on-line SPE–LC–MS, off-line SPE–LC–MS and LC–MS

	Detection limit (ng l^{-1})				
	CQ	MQ	DQ	PQ	DF
On-line SPE–LC–ESI-MS	20	10	50	60	5
Off-line SPE–LC–ESI-MS[a]	100	100	900	4700	50
LC–ESI-MS	900	400	6000	11 000	100

[a] Ref. [32].

adjust the sample volume in order to obtain a very good recovery for all the compounds. Moreover, although it is generally assumed that it is better to work with a 100% recovery, for trace analysis the most significant point is the amount available for detection, which has to be as high as possible for most of the compounds even if for some of them the recovery is not very high [28]. So, for the preconcentration study a volume of 50 ml was chosen. The recoveries for MQ, CQ and DF ranged between 70 to 84%, while a recovery of 87% was obtained for PQ and 92% for DQ. Fig. 2 shows the chromatogram obtained by percolating 50 ml of a solution of quats in water at a concentration level of 500 ng l^{-1} at the established conditions.

3.2. Quality parameters

In order to evaluate the performance of the on-line trace enrichment procedure coupled to LC–MS, the figures of merit were studied. Day-to-day and run-to-run precisions were determined by preconcentrating 50 ml of a water solution of 250 ng l^{-1} and quantifying using calibration curves constructed by preconcentrating water standard solutions between 50 and 400 ng l^{-1}. The run-to-run precision of the on-line extraction method was determined by six replicates carried out on the same day and under optimum conditions. Relative standard deviations (RSDs) in the range of 7 to 13% were obtained. The day-to-day precision was established with six replicates carried out on three different days. The RSDs were between 9 and 14%. The values obtained for the run-to-run precision were higher than those obtained for the direct injection (between 6 to 8%), but slightly lower than the RSDs obtained with the off-line procedure (between 8 and 14%). The same behavior was observed with the day-to-day precision. Also good run-to-run (from 1 to 3%) and day-to-day (from 2 to 9%) accuracies were obtained. These results confirm that a good precision can be attained with the on-line preconcentration procedure described above.

The LODs, expressed as ng l^{-1} and based on a signal-to-noise ratio of 3:1, were estimated in HPLC-grade water spiked at low levels. These limits ranged from 5 to 60 ng l^{-1} (Table 1). As can be seen, the on-line preconcentration step produced a significant improvement in the detection limits, which were lower than those obtained by direct injection and by the use of the off-line procedure [32]. Moreover, the LODs obtained are lower than those reported by other authors using SPE and LC–UV diode array detection (400 to 800 ng l^{-1} for PQ and DQ) [20] or LC–ESI-MS (100 to 200 ng l^{-1} for DQ and PQ) [16]. The LODs obtained for the on-line procedure

Table 2
Determination of quats in a drinking water sample using on-line SPE–LC–MS

	Expected value (ng l^{-1})	External calibration		Standard addition	
		(ng l^{-1})	RSD (%)	(ng l^{-1})	RSD (%)
CQ	121	190	9	130	8
MQ	129	210	11	132	10
DQ	99	100	10	112	13
DF	126	130	8	130	9

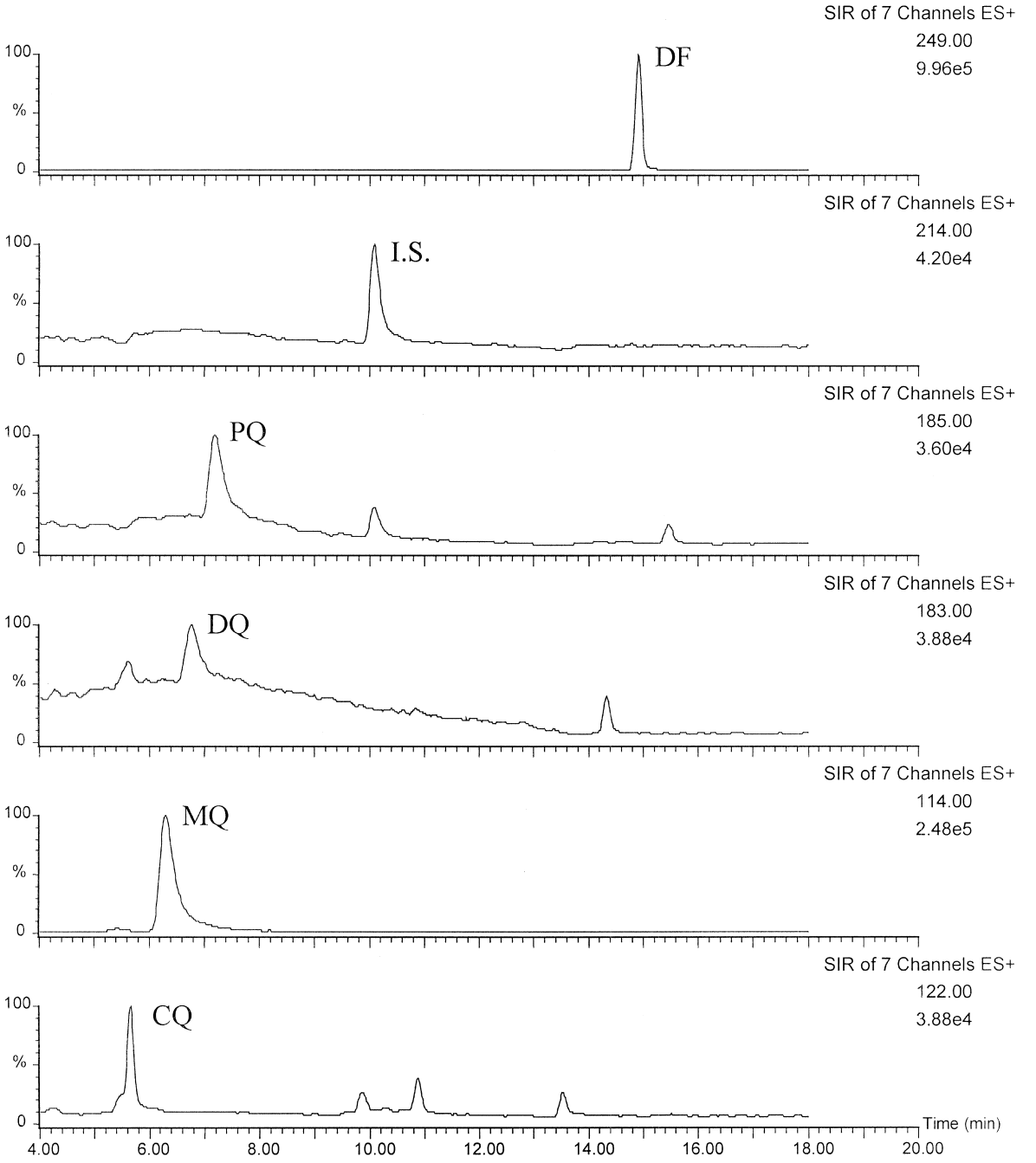

Fig. 3. On-line SPE–LC–ESI-MS chromatogram obtained for the on-line preconcentration of a water sample spiked at a concentration level of 100 ng l^{-1}. MS: acquisition data in SIM mode. Time scale in min.

ensure compliance with the European Community directives for drinking water.

3.3. Determination of quats in drinking water samples

In order to establish the applicability of the method, a drinking water sample was analyzed. A drinking water sample free of quats, was spiked at the maximum admissible level established by the European Community directive, 100 ng l^{-1}, treated as described in Section 2.4 and analyzed in duplicate. Quantitative analysis was carried out using two methods, external calibration and standard addition and results were compared. For the external calibration method, curves were constructed using HPLC-grade water standard solutions from 50 to 400 ng l^{-1}, while standard addition was carried out by adding to the spiked drinking water sample, a standard solution at five concentration levels between 50 to 400 ng l^{-1}. Since no matrix interferences were detected in the non-spiked water samples, the detection limits estimated (from 6 to 85 ng l^{-1}) were similar to those obtained by preconcentrating standard solutions. The values obtained for the quantitative analysis of the spiked sample are given in Table 2, where it can be observed that more accurate results were obtained using the standard addition method than that of the external calibration although the precision is similar for both methods. Fig. 3 shows a chromatogram obtained for the on-line preconcentration of a water sample spiked at a concentration level of 100 ng l^{-1}.

4. Conclusion

On-line solid-phase extraction using C_8 extraction disks and liquid chromatography coupled to mass spectrometry using the preconcentration of only 50 ml of water has been shown to constitute a good approach for the analysis of quats in water samples at concentrations below 100 ng l^{-1}. Quality parameters have shown that good precision, accuracy and low detection limits can be obtained providing an easy and rapid procedure to determine quats for water monitoring purposes. The method was applied to drinking waters and it is able to quantify the amounts established by the European Union directive.

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