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Journal of Chromatography A, 1050 (2004) 179–184
JOURNAL OF CHROMATOGRAPHY A
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Application of internal quality control to the analysis of quaternary ammonium compounds in surface and groundwater from Andalusia (Spain) by liquid chromatography with mass spectrometry☆

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Received 8 March 2004; received in revised form 17 July 2004; accepted 11 August 2004

Abstract

A method has been developed for the simultaneous determination of paraquat (PQ), deiquat (DQ), chlormequat (CQ) and mepiquat (MQ) in water samples by liquid chromatography (LC) coupled with electrospray ionization mass spectrometry (MS). The LC separations of the target compounds, as well as their MS parameters, were optimized in order to improve selectivity and sensitivity. Separation was carried out in a Xterra C_8 column, using as mobile phase methanol–heptafluorobutyric acid (HFBA) in isocratic mode. The molecular ion was selected for the quantitation in selected ion monitoring (SIM) mode. Off-line solid-phase extraction (SPE) was applied with silica cartridges in order to preconcentrate the compounds from waters. Detection limits were in the range 0.02–0.40 $\mu g\,l^{-1}$. Recovery range varied between 89 and 99.5% with precision values lower than 6%. The method has been applied successfully to the analysis of both surface and groundwater samples from agricultural areas of Andalusia (Spain), using well defined internal quality control (IQC) criteria. The results revealed the presence of deiquat and paraquat in some samples.

Keywords: Quaternary ammonium compounds; Groundwater; Surface water

1. Introduction

Herbicides and plant growth regulators are essential components of modern agriculture in developed countries and their use is increasing in the third world countries. In agricultural areas, large quantities of these compounds are used, most of them applied directly to the soil or sprayed over the crop fields. In consequence, they can enter as contaminants into natural waters, soils or food. We have focused this study in a particular group of quaternary ammonium compounds for their interest, the paraquat (PQ) and deiquat (DQ) herbicides, and the chlormequat (CQ) and mepiquat (MQ) plant growth regulators. PQ and DQ are non-selective contact herbicides used for the control of weeds and grasses in fruit orchards and for control of aquatic weeds. CQ is registered in at least 17 countries as a plant growth regulator particularly to promote sturdier growth in wheat, rye and oats and thus reduce the risk of lodging. MQ is a plant growth regulator used on cotton to inhibit sprouting [1].

Quaternary ammonium pesticides are commonly known as quats. These compounds are polar and with a high water solubility. So, it is necessary to keep in mind that some of these compounds can arrive easily until the supplies of water. Their ionic character provides them a great mobility in the environment that it is fundamental to control its possible presence in the next places to aquatic supplies. Due to the form in that these substances affect to the environment, some of them have been included on priority lists and are currently regulated in a number of countries [2]. The European Union has not regulated the levels of these compounds in drinking

☆ Presented at the 3rd Meeting of the Spanish Association of Chromatography and Related Techniques and the European Workshop: 3rd Waste Water Cluster, Aguadulce (Almeria), 19–21 November 2003.

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doi:10.1016/j.chroma.2004.08.023

water and continues applying the values of 0.1 μg l^{-1} for individual pesticides and 0.5 μg l^{-1} for total pesticides [3].

The analysis of quats in water is difficult. Due to their properties, an ion-pair can be used to be determined by liquid chromatography (LC) with UV detection [4,5]. However, in order to increase the sensitivity and selectivity, LC coupled with mass spectrometry (MS) is the preferred technique for the determination of these compounds using thermospray [6], particle beam [7], electrospray (ESI) [8–11] or atmospheric pressure chemical ionization [10]. Because of the large-scale dilution of contaminants in the aquatic matrices, concentrations of many pesticides are below the detection limits of standard analytical and sampling methods. This makes necessary the use of enrichment methods to comply with the maximum legally permitted levels of quats in water samples. Solid-phase extraction (SPE) is a versatile and successfully applied technique in environmental analyses, which has often been recommended for the isolation and concentration of quaternary ammonium herbicides [12]. Cation-exchange resins [7,13,14], and other fases such as silica [10,15–17] or graphitic carbon [16,18] have been used to concentrate quats from waters.

In this work, a SPE method with silica cartridges, in combination with LC–MS, has been developed for the determination of four quaternary ammonium compounds in environmental water samples, both surface and groundwater. The methodology was applied to the analysis of real samples from Andalusia (South of Spain), in order to know the pollution levels with these compounds. The study was mainly applied to waters used in rise or olive-tree fields in which the presence of humid acids, surfactants, inorganic salts, other pesticides or related compounds such as metabolites could diminish the recovery and interfere in the instrumental step [19,20]. Internal quality control (IQC) criteria were observed in the analysis of samples in order to ensure the reliability of the obtained results.

2. Experimental

2.1. Chemicals and solvents

Chlormequat chloride (98.9%), mepiquat chloride (98.65%), paraquat dichloride hydrate (99.9%), and deiquat monohydrate were purchased from Riedel de Haen (Seelze, Gremany). All these quaternary ammonium compounds were used for preparing 200 mg l^{-1} stock standard solutions in water–methanol (1:1) and were prepared in plastic material to prevent adsorption and stored 4 °C in the dark. NaOH (99.5%) and HCl 37% were purchased from Panreac (Barcelona, Spain). Heptafluorobutyric acid (HFBA) was obtained from Sigma (Poole, UK). Methanol and acetonitrile were HPLC grade gradient from Panreac. Milli-Q was used from apparatus Millipore (Bedford, MA, USA). Sep-Pak cartridges of silica (500 mg) and Oasis HLB (200 mg) were purchased from Water (Milford, MA, USA). Discovery DSC-CN SPE cartridge (500 mg) and discovery DSC-WCX SPE (500 mg) were purchased from Supelco (Bellefonte, PA, USA).

2.2. Apparatus

HPLC system was an Alliance 2695 equipped with autosampler, degasser, and heater column purchased by Water. Mass spectrometer system was a ZQ 2000 single quadrupole purchased by Water-Micromass (Manchester, UK). Data were collected by MassLynx 4.0 software in a personal computer. The compounds were carried out by a Xterra C_8 150 mm × 2.0 mm i.d., 5 μm from Waters with a isocratic method of 5% methanol and 95% HFBA 20 mM with a flow of 0.2 ml min^{-1}. The column temperature was set to 30 °C during all running and the injection volume was 20 μl.

Analytes were detected with electrospray probe in positive mode. The temperature source was 130 °C and the flows for desolvation and cone gas were 300 l h^{-1} and 50 l h^{-1}, respectively, and the capillary was set to 3.5 kV. Selected ion monitoring (SIM) were acquired by previous infusion of each standard in full scan mode at concentration of 15 mg l^{-1}.

2.3. Sampling

Samples were collected in 1 l amber glass bottles capped with teflon lined screw caps. Replicated samples were taken. After filling with water, the bottles were shaken vigorously for 1 min, and iced down in the field and kept refrigerated at 4 °C away from light prior to extraction, which was done within 48 h. The extracts were analyzed before two weeks of collection. Handling avoided the contamination of the samples.

Forty-one samples were taken from different wells or surface waters affected by agricultural development (Fig. 1). One of these samples (no. 21) was taken from a deep well (367 m). This one was chosen as field blank.

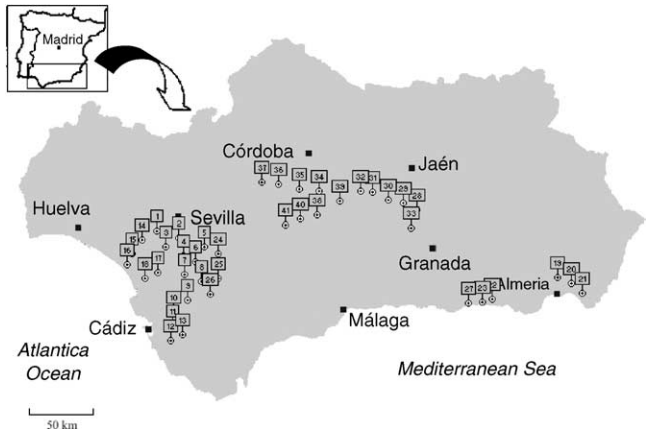

Fig. 1. Area studied and location of sampling points.

Table 1
MS conditions and validation parameters of the LC–MS method

Pesticide	*m/z*	Voltage cone (V)	Recoveries (%)	R.S.D. (%)	RTW (min)	r^2	LOD ($\mu g\,l^{-1}$)	LOQ ($\mu g\,l^{-1}$)
CQ	122/124	20/20	99.5	4.8	3.85–3.99	0.98	0.02	0.05
DQ	183/157	20/50	98.7	5.6	4.80–4.90	0.99	0.50	2.00
MQ	114/98	20/60	88.9	3.9	5.37–5.48	0.99	0.02	0.05
PQ	185/171	30/30	92.8	5.9	5.55–5.67	0.99	0.40	1.00

2.4. Extraction procedure

An aliquot of 250 ml of water samples was adjusted to pH 9 with NaOH and passed through a silica cartridge without condition at a flow rate of 3–4 ml min^{-1}. The column material was not allowed to become dry. The elution was made with 5 ml of 6 M HCl–MeOH (9:1, v/v). The eluate was evaporated to dryness under a stream of N_2, and in order to favor this step the eluate was immersed in hot water. The final residue was filled up to 1 ml with mobile phase (20 mM HFBA).

3. Results and discussion

Until now, some analytical methods have been published for determining quaternary ammonium herbicides in waters using silica cartridges [10,21,22]. Also, different LC–MS methods have been described for their analysis [8–11]. However, few references have been found to the use of silica cartridges in combination with LC–ESI-MS [10]. The LC–MS method described in this work is faster (analysis time lower than 6 min) and more precise than the previously published [10]. Another advantage of the current method was that peak tailing did not occur.

3.1. Optimization and validation of the LC–MS method

Ionization and fragmentation conditions were optimized for the pesticides by continuous flow injection of pure standard solutions of 15 mg l^{-1} in methanol–20 mM HFBA (5:95, v/v). The best response was obtained by electrospray ionization using positive mode. Table 1 summarises the optimised MS conditions.

Different mobile phases were tested in order to separate the target compounds. After the optimization, the separation was carried out with a isocratic method using as

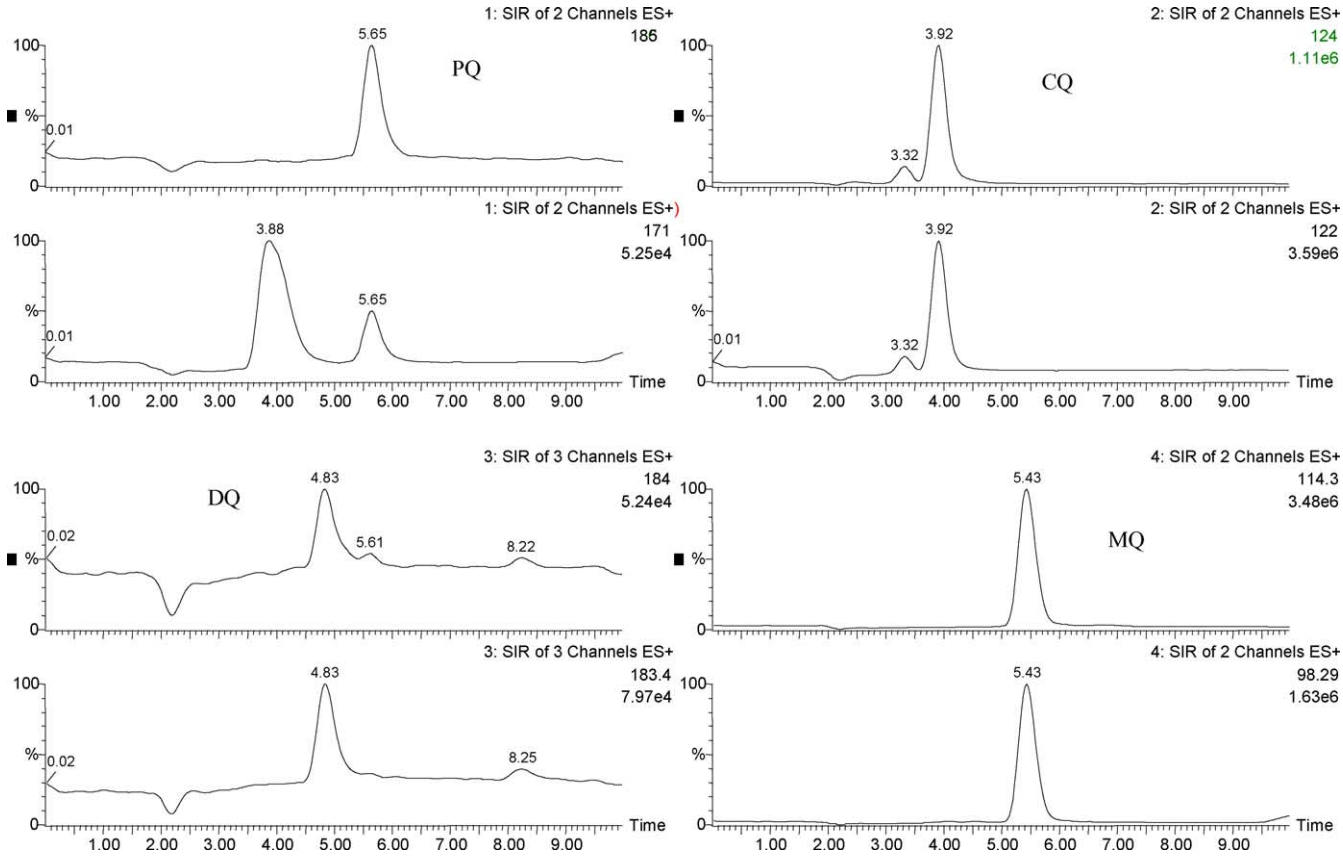

Fig. 2. LC chromatogram of the groundwater sample taken as field blank fortified with 25 $\mu g\,l^{-1}$ of a standard solution of the pesticides.

mobile phase methanol–20 mM HFBA (5:95) with a flow of 0.2 ml min^{-1} (Fig. 2). A good separation among analytes was reached, which is suitable for obtaining accurate calibrations. This situation was also adequate for the resolution among the analytes and potential interferent compounds, as well as other pesticides used in this area or background interferences co-extracted from complex environmental water samples.

Due to the polarity of these compounds, SPE was tested with different sorbent materials, like Oasis HLB (polydivinylbenzene–*N*-vinylpyrrolidone copolymer), DSC-CN (cianopropyl endcapped) and DSC-WCX (ion-exchange cartridge, propyl carboxi phase) and silica cartridges, and at several pH values in the water samples. The HLB, DSC-CN and DSC-WCX cartridges were preconditioned, each one of them, by passing 5 ml methanol followed by 4 ml of water. For HLB cartridges, an ion-pair (100 μl HFBA) was added to the water samples. The elution of quats from HLB cartridges was made with 5 ml of MeOH and 5 ml of MeOH–10% NH_4Cl (1:9). After that, the extract was evaporated to dryness under hot water and N_2. Then, mobile phase was added to a final volume of 1 ml. The recoveries of CLQ and MQ were close to 100% but PQ and DQ were not found. DSC-CN and DSC-WCX cartridges were eluted with 5 ml of 6 M HCl–10% MeOH, and results similar to those obtained with the HLB cartridges were achieved. In order to improve these results, silica cartridges were also tested. It is known that silica cartridges have the capacity of cation exchange that increases in basic pH. So, water samples were adjusted to pH 9. The elution of quats was similar to that of the DSC-CN and DSC-WCX cartridges. Now, satisfactory results were obtained for all the target compounds.

All validation experiments were performed taking into account the extraction procedure. The identification of the target pesticides was carried out by searching in the appropriate retention time windows (RTWs), retention time average ± 3 standard deviations of the retention time of 10 blank samples spiked at a mid-level calibration standard for each compound (Table 1). The confirmation of a previously identified compound was done by ion ratio obtained from the ratio of the concentration of two ions for the same compound. For a confirmation of the result, the ion ratio value obtained must be between 80 and 120% for each compound.

The linearity of the method was studied by extraction of blank samples spiked with the compounds at four different concentration levels, in the range 0.05–10 μg l^{-1} for CQ and MQ, 2–100 μg l^{-1} for DQ and 1–100 μg l^{-1} for PQ. Linear calibration graphs were constructed by least-squares regression of concentration versus peak area and peak height of the calibration standards. Slightly better results were achieved using relative areas for all compounds. Good linearity was found in the concentration range tested, with determination coefficients (r^2) higher than 0.98 in all the cases. Detection (LODs) and quantification (LOQs) limits of target analytes were determined as the lowest fortification level that yielded a signal-to-noise (S/N) ratio of 3 and 10 (when the quantification ion was monitored), respectively. They were established spiking Milli-Q water samples with increasing concentration levels of the compounds. The LODs obtained for these compounds were below 0.5 μg l^{-1} (Table 1).

Three aliquots of 250 ml of Milli-Q water spiked with 5 μg $l^{-1}$of CQ and MQ, and 50 μg l^{-1} of DQ and PQ were used to study the extraction efficiency of the analytes using the calibration methods previously described and good recoveries (88.9–99.5%) and precision values (3.9–5.9%, expressed as relative standard deviation) were obtained for all the compounds (Table 1).

In comparison with previously published LC–MS methods in combination with off line SPE, our method shows better precision values, lower analysis time and similar recovery and lower limits.

3.2. Internal quality control

Internal quality control (IQC) has to be applied when analysing samples to ensure reliable results, because it allows the monitoring of the process in order to assure that it is under statistical control and that the variability of the results is included in the range established by the uncertainty of the method. IQC must be observed in the identification, confirmation and quantification steps. IQC criteria must be applied after analysing a certain number of samples. We considered 20 samples as the right size of the batch because we did not observed drift in the measurement chemical process during the corresponding period of time. Laboratory reagent blank and field reagent blank allowed the analyst to ensure that the analytical signal is not affected by contamination and is attributable just to the analyte [23,24]. Reference materials were used to study the variation between batch of samples, verifying the comparability of the measures. We used a home-made reference material, spiking a sample of water with the target analytes. This reference material was stored and checked for stability and homogeneity and then used as a quality control sample that was analyzed with every batch of samples. The variations in the data obtained from the quality control sample were monitored on a quality control chart [25–27]. Duplicated samples and blind samples provided a less formal means for checking of drift than quality control samples. They were analyzed (1 every 10 samples) to check intra-batch precision. IQC included the use of a minimum of three standard concentrations of the calibration for each analyte. The slopes of the different calibration graphs must not differ significantly from the value found at the stage of validation of the method. The slopes were compared with that obtained in the validation of the analytical method because a decrease in the slope value may be critical when working with values close to the LOQ. Recoveries were measured for each batch of samples to check the performance of the MCP. Routine recovery values are acceptable in the range 70–130% [28]. Finally, retention time windows was used as

identification criterium and the inter-ion abundance ratios of two ions, obtained for a calibrating standard of the compound analyzed under the same conditions, was used as confirmation criterium.

3.3. Application to environmental water samples

Once the analytical methodology was validated, it was applied to the analysis of 40 environmental samples, 25 groundwater and 15 surface water samples collected from agricultural areas in Andalusia (South Spain). In this region, pesticides and herbicides are widely used to protect crops being normally applied to soil or to plant. The set of samples analyzed each day was processed together with: (i) analysis of a field blank extract that eliminates a false positive by contamination in the extraction process; (ii) analysis of a field blank spiked at the concentration of the second calibration level in order to assess the extraction efficiency; real samples were analyzed if recoveries were between 70% and 120%; (iii) 10% of duplicated and blind samples; and (iv) calibration curves prepared daily to check both, sensitivity and linearity.

Pesticides were detected in 24 of the 40 samples, generally at concentrations below their LOQ (Table 2). DQ and PQ were the compounds more frequently detected; they were found in 15 and 13, respectively, of the 40 samples ranging from 1–42 $\mu g\,l^{-1}$ for DQ to 2–12 $\mu g\,l^{-1}$ for PQ. Fig. 3 shows a positive of DQ as an example of a real surface sample analyzed.

Table 2
Positive identifications ($\mu g\,l^{-1}$) of pesticides in the analyzed groundwater samples

Sample	Chlormequat	Deiquat	Mepiquat	Paraquat
1	–	<LOQ	–	<LOQ
2	–	2	–	5
3	–	<LOQ	–	2
4	–	10	–	<LOQ
5	–	–	–	–
6	–	–	–	<LOQ
7	–	42	–	–
8	–	5	–	–
9	–	<LOQ	–	3
10	–	2	–	–
14	–	–	–	<LOQ
15	–	<LOQ	–	–
17	–	<LOQ	–	–
24	–	3	–	–
25	–	1	–	–
26	–	–	–	12
28	–	–	–	2
30	–	<LOQ	–	–
33	–	–	–	<LOQ
34	–	–	–	<LOQ
36	–	<LOQ	–	–
37	–	<LOQ	–	–
38	–	–	–	3
40	–	–	–	<LOQ

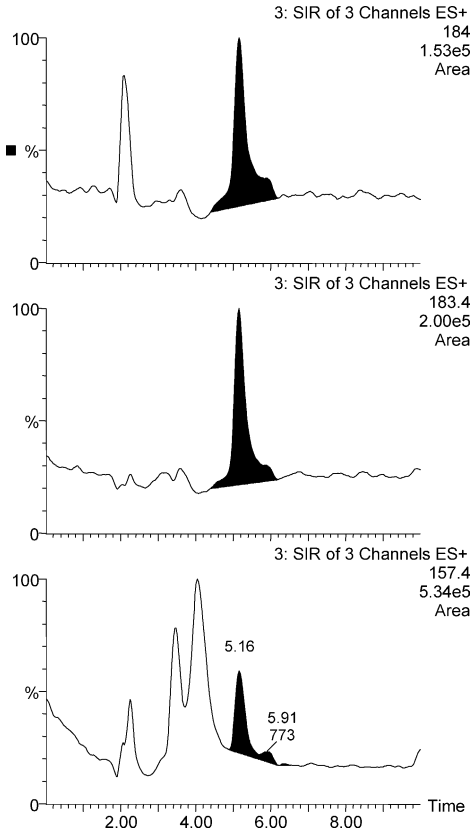

Fig. 3. DQ chromatogram in the surface water sample 7. Concentration found 42 $\mu g\,l^{-1}$.

4. Conclusions

Off-line SPE and LC–ESI-MS using the preconcentration of 250 ml of water has been shown to be a good approach for the analysis of paraquat, diquat, chlormequat and mepiquat in water samples. Silica cartridges have showed the best results for the extraction of the target compounds. Validation parameters have shown a good accuracy, precision and lower limits to monitor quats in environmental waters. IQC were established for analysing samples. The method was applied to the analysis of 40 environmental water samples from Andalusia (South Spain). Pesticides were detected in the 60% of the samples. This study clearly demonstrates that the great use of pesticides in agricultural areas from Andalusia has affected the water quality with respect to content of pesticides. For that, it is evident the need of monitoring water quality with respect to content of pesticides in this area.

Acknowledgements

The authors are very grateful to Framework Agreement between the University of Almería and the Consejería de Medio Ambiente de la Junta de Andalucía, and also with the Consejería de Educación y Ciencia (Research

program ORTI's, empresas andaluzas 2003) de la Junta de Andalucía.

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