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Journal of Chromatography A, 961 (2002) 65–75

JOURNAL OF CHROMATOGRAPHY A

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Analysis of the herbicides paraquat, diquat and difenzoquat in drinking water by micellar electrokinetic chromatography using sweeping and cation selective exhaustive injection

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Abstract

Optimum conditions for the determination of the herbicides paraquat, diquat and difenzoquat by micellar electrokinetic chromatography (MEKC) using sweeping and cation-selective exhaustive injection (CSEI) as on-line concentration methods were developed. Sodium dodecyl sulfate (80 m*M*) in 50 m*M* phosphate buffer (pH 2.5) with 20% acetonitrile was used as a background electrolyte for the methods studied. The limits of detection, based on a signal-to-noise ratio of 3:1, were about 2.6–5.1 mg l^{-1} in purified water when MEKC was applied for the standards. By using an on-line preconcentration method known as sweeping–MEKC, up to a 500-fold increase in detection sensitivity was obtained whereas up to a 50 000-fold increase for CSEI–sweeping–MEKC was achieved. The limits of detection using optimum CSEI–sweeping–MEKC were lower than 1 μg l^{-1} and the method was validated obtaining good reproducibility (relative standard deviation lower than 22%) and linearity. CSEI–sweeping–MEKC was successfully applied to the determination of the three herbicides in spiked tap water below the levels established by the US Environmental Protection Agency.

Keywords: Water analysis; Sweeping; Injection methods; Cation-selective exhaustive injection; Sample stacking; Paraquat; Diquat; Difenzoquat; Pesticides; Quaternary ammonium compounds

1. Introduction

The widespread use of pesticides and herbicides is an essential component of modern agriculture to boost productivity. However, the increasing concern about environmental pollution and drinking water and food contamination has led to the establishment of strict regulations that have driven efforts to develop highly sensitive analytical methods [1]. Quaternary ammonium herbicides constitute a particularly difficult group of herbicides to analyze, commonly known as ''quats''. Paraquat (PQ) and diquat (DQ) are used as non-selective contact herbicides for the control of weeds and grasses in plantation crops, for pasture renovation and as defoliants for cotton and hops. Difenzoquat (DF) is a selective herbicide used for post-emergence control of wild oats in cereal crops [2]. On the basis of their toxicity, the World Health Organization (WHO) has classified these three compounds as moderately hazardous [3]. Given the threat they pose to the

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0021-9673/02/$ – see front matter
PII: S0021-9673(02)00031-6

environment, some of these compounds have been included on ''priority'' lists and are currently regulated in a number of countries [4,5]. For drinking waters, the Office of Water of the US Environmental Protection Agency (EPA) 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 [6,7]. The European Union has not regulated the levels of these compounds in water and the values 0.1 $\mu g\ l^{-1}$ for individual pesticides and 0.5 $\mu g\ l^{-1}$ for total pesticides are applied [8]. To help enforce the legislated values, sensitive analytical methods for quats still need to be developed.

The analysis of these compounds is difficult due to their cationic character. Nevertheless, a great number of ion-selective [9–11], spectrophotometric [12,13], spectrofluorimetric [14] and enzyme-linked immunosorbent assay (ELISA) [15,16] methods have been developed. Moreover, these compounds are polar, easily soluble in water and have low volatility. Because of these properties, they are usually determined by ion-pair high-performance liquid chromatography with direct UV detection [17,18]. In order to improve sensitivity and selectivity, liquid chromatography coupled to mass spectrometry (LC–MS) has also been used [19–23].

Capillary electrophoresis (CE) has also been demonstrated to be a promising alternative for the separation and/or analysis of quaternary ammonium herbicides. Quats have been determined by CE in water [17,24–27] and in other matrices such as serum, soil and urine [28,29]. CE coupled to mass spectrometry (CE–MS) has also been applied to analyze quats in water samples [30–32]. Nevertheless, CE has lower concentration sensitivity than high-performance liquid chromatography due to both the low sample injection volume and the short optical path-length for on-capillary detection. To detect the maximum legally permitted levels of quats in drinking water [6–8], enrichment procedures prior to determination have to be used in order to enhance sensitivity. Off-line preconcentration procedures have been used to attain low detection limits and have been applied to the analysis of quats in drinking water [27,33] but usually involve long analysis times and laborious sample handling. Several techniques for on-line preconcentration have been reported for the analysis of these compounds. Isotachophoresis (ITP) has been used as an on-line water sample pre-treatment for the determination of PQ and DQ by CE [34]. A sample stacking procedure has been reported for the analysis of quats in drinking water [35]. This method involves field polarity reversal after the capillary has been filled with a large volume of sample of lower conductivity than the buffer used for CE separation. However, it was only appropriate for the analysis of highly contaminated water samples due to limited sensitivity, so other on-column preconcentration methods are needed.

Micellar electrokinetic chromatography (MEKC) has become widely popular as a powerful separation technique for both neutral and ionic compounds, as first introduced by Terabe et al. [36]. The separation mechanism involves differential partitioning of analytes between the pseudostationary phase (micelles) and the surrounding aqueous phase and their electrokinetic transport. In order to improve concentration sensitivity in MEKC, different on-line concentration methods have recently been reported. One of these methods was first introduced by Quirino and Terabe [37] and is referred to as sweeping. It consists in the introduction of a large sample zone prepared in a matrix devoid of pseudostationary phase, wherein the analytes are picked-up and accumulated by the pseudostationary phase that penetrates the sample zone. Anionic surfactants have been used in most of the papers that deal with on-line sample concentration for MEKC [38,39]. Different studies using sweeping–MEKC with cationic micelles under strong electroosmotic flow (EOF) conditions and suppressed EOF conditions have also been reported [40,41] with sensitivity enhancements of over a 1000-fold [40] for some analytes. Recently, a combination of sample stacking and sweeping, referred to as cation-selective exhaustive injection and sweeping (CSEI–sweep) has achieved almost a million-fold enhancement in detector response for cationic hydrophobic analytes [39]. On-line concentration of positively charged analytes with anionic sodium dodecyl sulfate (SDS) micelles provided high sensitivity enhancements because of the strong interaction between oppositely charged analytes and the SDS micelle [42].

In this paper, the conditions for the simultaneous determination of paraquat, diquat and difenzoquat by MEKC using both sweeping and CSEI–sweeping as on-line concentration are developed. Ethylviologen

(EV) and heptylviologen (HV) are used as internal standards. Quality parameters such as limit of detection, repeatability, inter-day reproducibility and linearity were assessed. Optimum CSEI–sweeping–MEKC was successfully applied to the analysis of quats in spiked tap water at the levels established by the US EPA.

2. Experimental

2.1. Instrumentation

A Hewlett-Packard ^{3D}CE System (Waldbronn, Germany) with a UV absorbance detector was used. Electrophoretic data were processed using a HP ^{3D}CE ChemStation software. An uncoated fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) of 60 cm (51.5 cm effective length)×50 μm I.D.×360 μm O.D. was used. The capillary was thermostated at 25 °C. Samples were introduced by pressure (5 kPa) or electrokinetic injection. Direct detection was performed at two wavelengths, 220 nm for DQ and 255 nm for PQ and DF. Electrophoretic separation was carried out using 80 m*M* SDS in 50 m*M* phosphate buffer (pH 2.5) with 20% acetonitrile as the background electrolyte (BGE). The BGE was filtered through a 0.45-μm membrane filter and sonicated before use. Conductivity of sample and separation solutions was measured using a Horiba ES-12 conductivity meter (Kyoto, Japan).

2.2. Chemicals

Diquat (1,1′-ethylene-2,2′-bipyridinium ion) and difenzoquat (1,2-dimethyl-3,5-diphenylpyrazolium ion) were purchased from Chemservice (West Chester, PA, USA) and paraquat (1,1′-dimethyl-4,4′-bipyridinium ion) from Sigma (St. Louis, MO, USA). Ethylviologen (1,1′-diethyl-4,4′-bipyridinium ion, EV) obtained from Aldrich (Milwaukee, WI, USA) and heptylviologen (1,1′-diheptyl-4,4′-bipyridinium ion, HV) from TCI (Tokyo, Japan) were used as internal standards. Structures of the quaternary ammonium herbicides and internal standards are shown in Fig. 1. Methanol, acetonitrile, hydrochloric acid and SDS were purchased from Nacalai Tesque (Kyoto, Japan), phosphoric acid

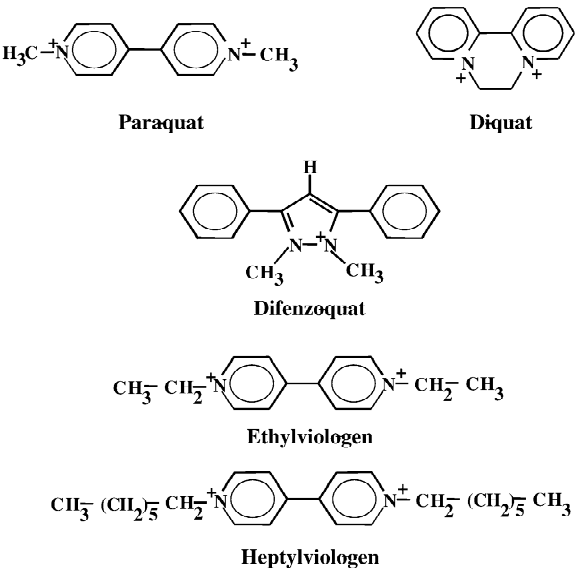

Fig. 1. Molecular structures of quaternary ammonium herbicides and internal standards.

solution (0.5 *M*) and sodium dihydrogenphosphate dihydrate from Kanto Chemical (Tokyo, Japan) and sodium hydroxide solution (1 *M*) from Wako (Osaka, Japan). Water used for the matrix and sample preparations was purified by using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Stock standard solutions of individual herbicides and internal standards (1 mg ml^{-1}) were prepared in purified water and stored in plastic vials to prevent adsorption. Working solutions were obtained by dilution with BGE, sample matrix or purified water, and were filtered through a 0.45-μm membrane filter before use. Buffers were prepared daily by dilution of stock solutions of phosphoric acid and sodium dihydrogenphosphate. Stock solutions of 0.5 *M* SDS were prepared every week in purified water. Micellar BGE were prepared (each day) by dilution of the SDS stock solution in appropriate phosphate buffers. Acetonitrile was added to the BGE to improve the separation. All buffers and working solutions were sonicated and filtered through a 0.45-μm membrane filter before use.

2.3. Capillary conditioning

New capillaries were pretreated by rinsing at

pressure (ca. 100 kPa) with 0.1 *M* hydrochloric acid for 15 min, water for 15 min, 1 *M* sodium hydroxide for 30 min and finally rinsed with water for 30 min. At the beginning of each day, the capillary was rinsed with 1 *M* sodium hydroxide for 10 min, followed by methanol for 10 min and then with water for 10 min. To ensure reproducibility, at the end of each run the capillary was treated with 1 *M* sodium hydroxide for 3 min, methanol for 3 min, water for 3 min and then with the BGE for 6 min. When the CSEI–sweeping–MEKC method was applied, the capillary was also treated at the end of each run and after the BGE with a non-micellar BGE for 4 min.

2.4. MEKC procedure

In the MEKC procedure, the sample was prepared directly in the BGE (80 m*M* SDS in 50 m*M* phosphate buffer at pH 2.5 with 20% acetonitrile). The capillary was first conditioned with the BGE and then, a conventional hydrodynamic injection of the sample (1 s, 5 kPa) was performed.

2.5. Sweeping–MEKC procedure

Sweeping–MEKC was performed according to the procedure reported by Quirino and Terabe [37] and Isoo et al. [43]. The capillary was first conditioned with a micellar BGE (80 m*M* SDS in 50 m*M* phosphate buffer at pH 2.5 with 20% acetonitrile). The sample prepared in a matrix (phosphate buffer at pH 2.5) having a conductivity similar to that of the BGE (~6.3 mS cm^{-1}) but devoid of micelles was injected hydrodynamically for 500 s (5 kPa). The injected analyte zone was assumed completely swept and the herbicides were separated by MEKC.

2.6. CSEI–sweeping–MEKC procedure

CSEI–sweeping–MEKC was performed following the procedure described by Quirino et al. [42]. The capillary was first conditioned with a nonmicellar BGE (100 m*M* phosphate buffer at pH 2.5, with 20% acetonitrile). A zone of a high-conductivity buffer (HCB) devoid of micelles (HCB, 200 m*M* phosphate buffer at pH 2.5) was hydrodynamically injected for 200 s at 5 kPa, followed by a 6-s injection (5 kPa) of a water plug. Then, the cationic herbicides prepared in a low-conductivity solution (purified water or tap water) were electrokinetically injected (400 s, +22 kV). The sample cations enter the capillary through the water plug with high velocities. Once the sample cations reached the interface between the water and HCB zones their velocities decreased due to the increase in the ionic strength and focus or stack at this interface. It should be noted that the EOF was suppressed by working in acidic conditions, so only the cations enter into the capillary when the positive voltage was applied. Then, the electrokinetic injection was stopped and micellar BGE solutions (80 m*M* SDS in 50 m*M* phosphate buffer at pH 2.5 with 20% acetonitrile) were placed at both ends of the capillary and the separation voltage (−22 kV) was applied. In this step, the anionic micelles will enter the capillary and sweep the previously stacked cationic herbicides and introduced the analytes as narrower bands. Finally, the herbicides were separated by MEKC.

3. Results and discussion

3.1. Micellar electrokinetic chromatography

As there is no previous work dealing with the determination of quaternary ammonium herbicides by micellar electrokinetic chromatography, a preliminary study was carried out using 50 m*M* SDS in 50 m*M* phosphate buffer (pH 2.5). A 100 mg l^{-1} mixture of the three herbicides and the two internal standards in the BGE was used. It should be noted that at pH 2.5, EOF was practically suppressed. When a negative polarity (−22 kV) was applied, the direction of the SDS micelles was toward the anode (detection window) interacting with the cationic quats and permitting their analysis. Under these conditions, the electropherogram obtained showed only two peaks due to partial comigration of these compounds. In order to improve the separation, the addition of an organic modifier to the BGE was necessary. For this purpose, different amounts of methanol or acetonitrile, from 5 to 20%, were added to the BGE. Fig. 2 shows the electropherograms obtained at 255 nm for two mixtures of herbicides, each one with one internal standard, using BGE

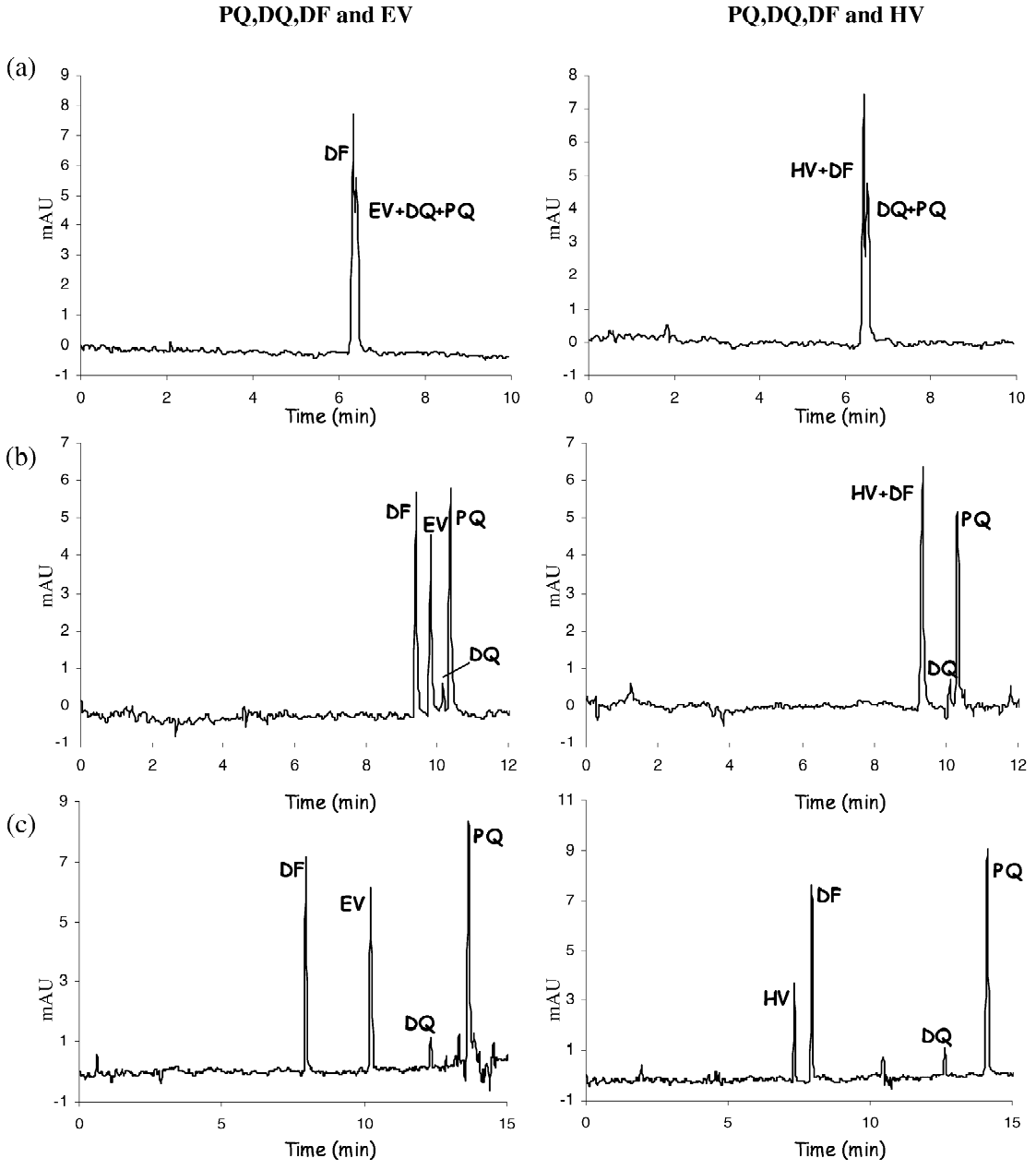

Fig. 2. Electropherograms obtained at 255 nm by MEKC for two mixtures of quats with each one of the internal standards (EV and HV). BGE: 50 m*M* SDS in 50 m*M* phosphate buffer (pH 2.5) (a) without organic solvent, (b) with 20% methanol, (c) with 20% acetonitrile.

without organic solvent (Fig. 2a), with 20% of methanol (Fig. 2b) and with 20% of acetonitrile (Fig. 2c). Both methanol and acetonitrile provided good electrophoretic separations of the three herbicides but only acetonitrile provided a good separation between the herbicides and both internal standards. Moreover, the resolution between PQ and DQ using 20% of acetonitrile in the BGE was higher than obtained with other electrophoretic methods [27,33,35].

Different SDS concentrations (50–100 m*M*) were

studied in order to improve the detection of the three herbicides. The BGE was 50 m*M* phosphate buffer (pH 2.5) with 20% acetonitrile. When high concentrations of SDS were employed, the resolution between HV and DF decreased and the baseline noise increased. The best separation and sensitivity were obtained when 80 m*M* SDS was added to the BGE and Fig. 3a shows the electropherograms at two wavelengths (220 and 255 nm) when a mixture of the five compounds studied were hydrodynamically injected (1 s, 5 kPa). Under these conditions, the complete separation of all five compounds was achieved showing that MEKC can be used for quat analysis.

The limits of detection (LODs) of quats using MEKC under optimal were 2.6, 3.2, and 5.1 mg l^{-1} for PQ, DF and DQ, respectively, based on a signal-to-noise ratio of 3:1. These LODs were similar to those obtained with other conventional electrophoretic separation techniques without using preconcentration methods [44].

3.2. Sweeping–MEKC

In order to increase the detection sensitivity, two different on-line preconcentration methods were investigated. The first was sweeping–MEKC. This procedure consists of a large hydrodynamic introduction of the sample solution into the capillary. The analytes must be prepared in a sample matrix with the same conductivity as that of the BGE but devoid of micelles. Different sample injection times (from 400 to 700 s, 5 kPa) were tested in order to obtain higher detector response. The best results were

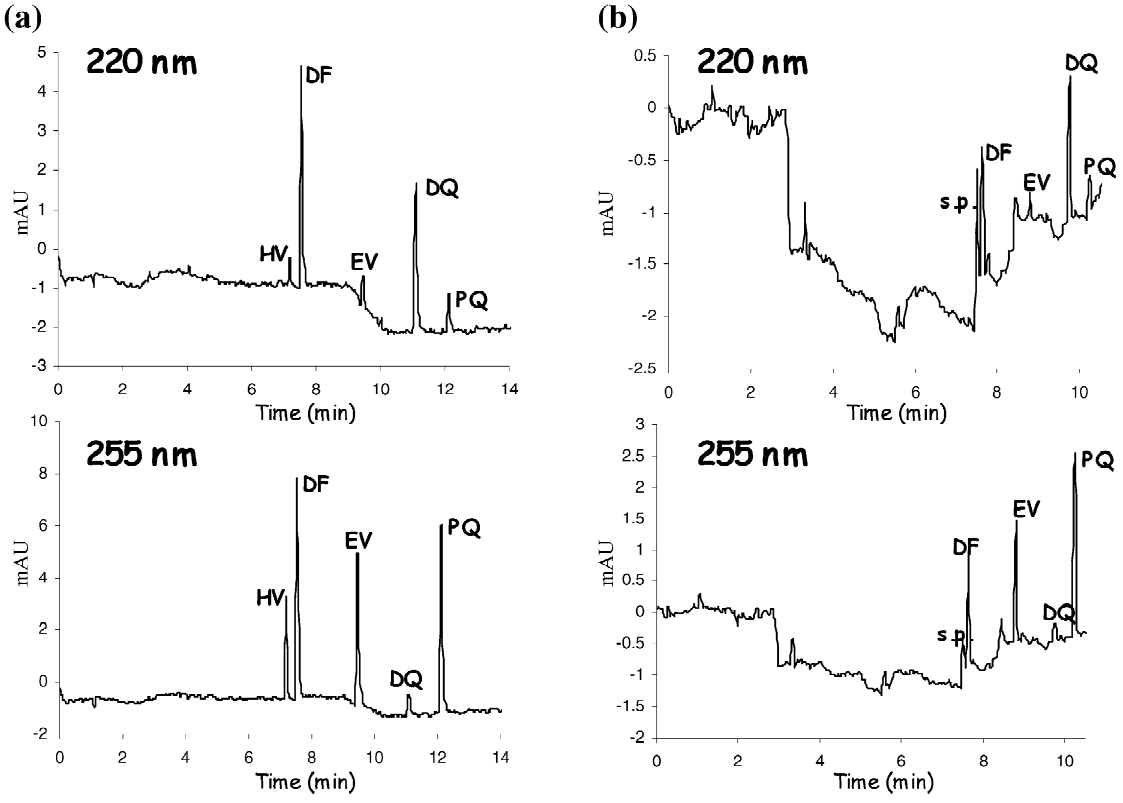

Fig. 3. Conventional MEKC and sweeping–MECK of quaternary ammonium herbicides. BGE: 80 m*M* SDS in 50 m*M* phosphate buffer (pH 2.5) containing 20% acetonitrile. (a) MEKC: Sample prepared in BGE; sample concentration, 100 mg l^{-1}; injection time, 1 s at 5 kPa. (b) Sweeping–MEKC: sample prepared in a phosphate buffer (pH 2.5) with the same conductivity of BGE (~6.3 mS cm^{-1}); sample concentration, 100 μg l^{-1}; injection time, 500 s at 5 kPa. Separation conditions (a and b): separation voltage, −22 kV with the micellar BGE at both ends of the capillary. s.p., system peak.

obtained for 500 s injection time. Fig. 3b shows the electropherograms obtained at two different wavelengths for a 100 μg l^{-1} mixture of PQ, DQ, DF, EV and HV by sweeping–MEKC. HV comigrated with a system peak that appeared close to DF preventing the use of this compound as internal standard. Higher sample injection times caused deterioration of the resolution between DF and the system peak and increased the baseline noise. Spikes in these electropherograms were caused by the change in composition of the liquid during sweeping [37]. Enhancement factors were calculated as the ratio of the peak heights obtained from sweeping–MEKC (Fig. 3b) and normal injection of 1 s by MEKC (Fig. 3a) and correction by the dilution factor (in this case 1000). The enhancement factors obtained were 230 for DF, 380 for DQ and 400 for PQ in terms of peak height.

3.3. CSEI–sweeping–MEKC

The enhancement obtained by sweeping–MEKC was insufficient to analyze quats in drinking waters at the legislated levels [6–8]. For this reason, other on-line concentration methods were studied. A new method that combine sample stacking and sweeping, CSEI–sweeping, achieved almost million-fold enhancements in detector response for some cationic hydrophobic analytes [39]. Thus, CSEI–sweeping was considered to be a promising method for the analysis of quaternary ammonium herbicides. In this case, the capillary was first filled with a nonmicellar BGE, because micelles interfered with the entry of the quats into the capillary when electrokinetic injection was used. Furthermore, sweeping can not occur in the resulting zone of electrokinetically injected quats. The concentration of phosphate buffer in this nonmicellar BGE was increased two-fold to compensate for the change in conductivity. Hence, 100 m*M* phosphate buffer pH (2.5) with 20% acetonitrile was used as nonmicellar BGE. After introduction of the nonmicellar BGE in the capillary, a high concentration phosphate buffer solution (HCB) devoid of organic solvent followed by a small water plug was hydrodynamically introduced into the capillary, before the electrokinetic injection of the sample. Fig. 4a shows the variation of the relative response of the three herbicides versus the

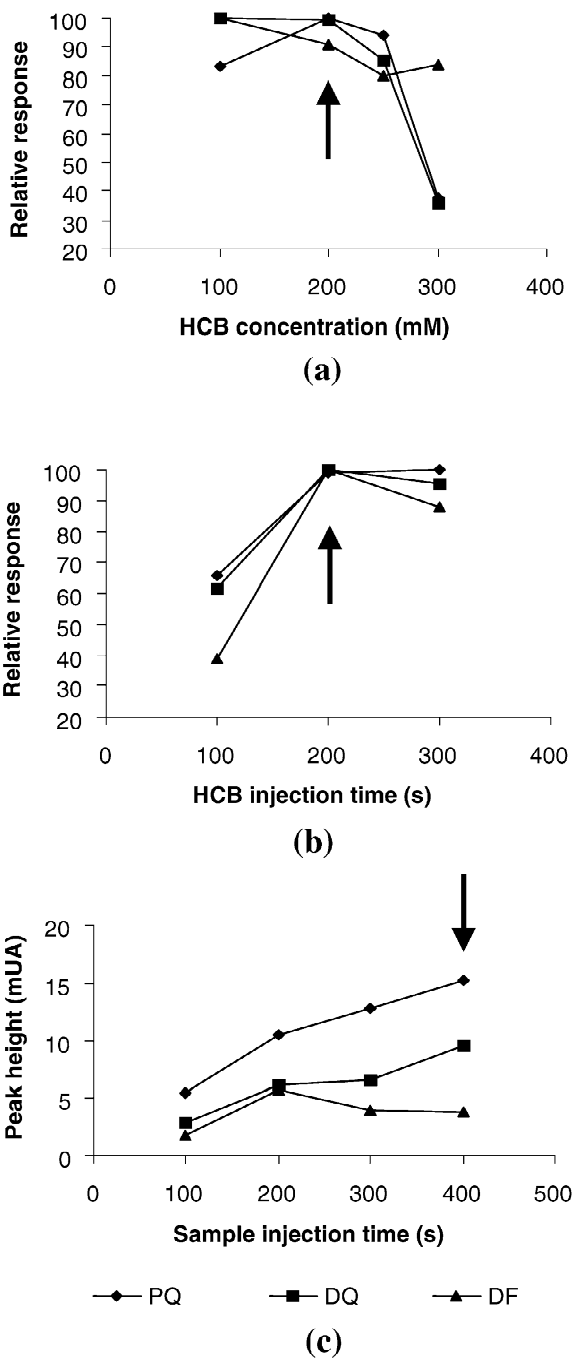

Fig. 4. (a) Effect of HCB concentration on the quaternary ammonium herbicide responses. (b) Effect of HCB injection time on the quaternary ammonium herbicide responses. (c) Variation of the herbicide peak heights versus the sample injection time.

HCB concentration. PQ and DQ gave the highest response when 200 m*M* phosphate buffer (pH 2.5) was used. A decrease in the response was observed at higher concentrations and this value has been chosen as optimal concentration. Fig. 4b shows the variation of the relative response of quats versus the hydrodynamic injection time of the HCB. For all three quats, higher response was obtained when 200 s was used. After the hydrodynamic injection of the HCB, a small water plug must be introduced into the capillary. Several groups have studied the effect of a water plug on sample stacking by electrokinetic injection [45,46] and Chien [45] has reported that a water plug provides a higher electric field at the tip of the capillary, which will eventually improve the sample stacking. If there is no water plug, the sample ions will stack at the injection point and cause degradation in the field enhancement. Thus, a 6-s hydrodynamic injection (5 kPa) of water followed by a large electrokinetic injection of sample at positive polarity was performed. Fig. 4c shows the peak height variation of quats with the sample electrokinetic injection time. For PQ and DQ the peak height increased with the injection time. Nevertheless, for DF a slight decrease at injections time higher than 200 s occurred. In order to obtain the highest signal for PQ and DQ (the only two quats legislated by the EPA [6,7]), 400 s electrokinetic injection was chosen as optimum injection time.

Fig. 5b shows the electropherograms obtained at

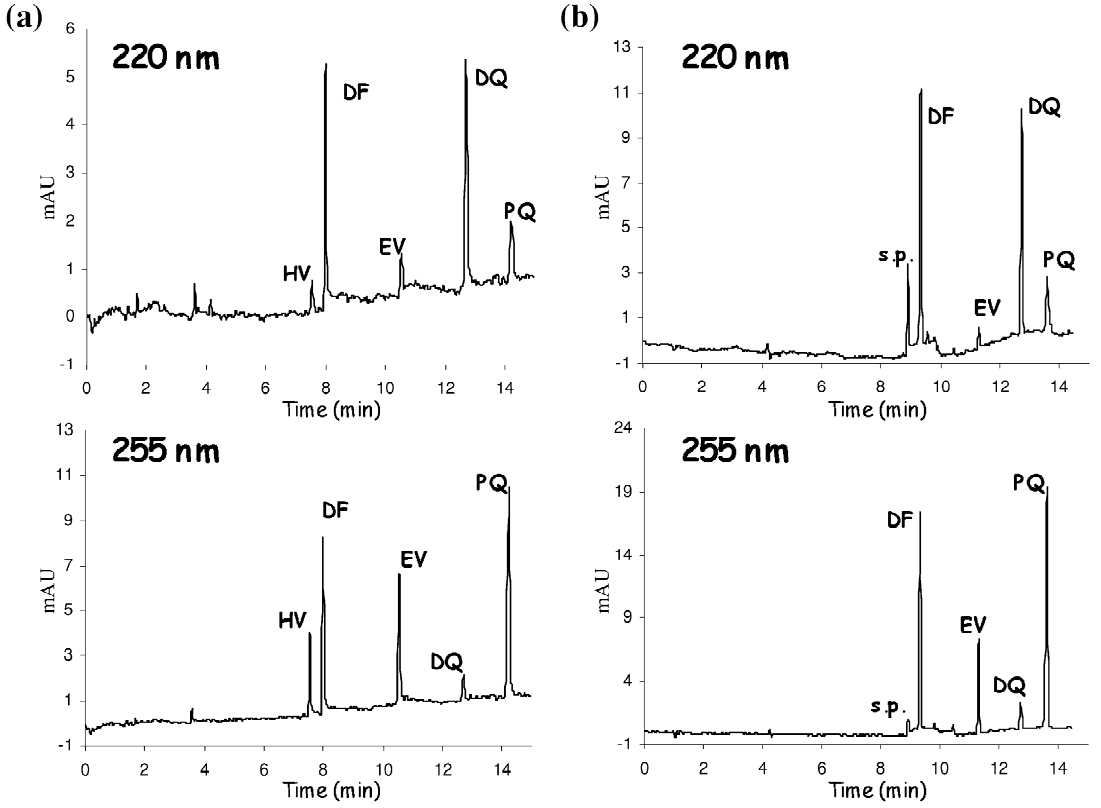

Fig. 5. Conventional MEKC and CSEI–sweeping–MEKC of quaternary ammonium herbicides. Nonmicellar BGE: 100 m*M* phosphate buffer (pH 2.5) containing 20% acetonitrile; micellar BGE: 80 m*M* SDS in 50 m*M* phosphate buffer (pH 2.5) containing 20% acetonitrile; HCB: 200 m*M* phosphate buffer (pH 2.5); conditioning solution before injection, (a) micellar BGE, (b) nonmicellar BGE; (a) MEKC: sample prepared in BGE; sample concentration, 100 mg l^{-1}; injection time, 1 s at 5 kPa. (b) CSEI–sweeping–MEKC: sample prepared in water; sample concentration, 10 μg l^{-1} PQ, DQ and EV, 50 μg l^{-1} DF. Injection scheme: hydrodynamic injection of HCB for 200 s (5 kPa), hydrodynamic injection of water for 6 s (5 kPa), electrokinetic injection of sample for 400 s (+22 kV); Separation conditions (a and b): separation voltage, −22 kV with the micellar BGE at both ends of the capillary. s.p., system peak.

two wavelengths for a 10 μg l^{-1} mixture of PQ, DQ, EV and HV and 50 μg l^{-1} of DF in purified water using CSEI–sweeping–MEKC under optimal conditions. HV comigrated with a system peak that appeared close to DF preventing the use of this compound as internal standard. Enhancement factors were calculated by the ratio of the peak heights obtained from CSEI–sweeping–MEKC (Fig. 5b) and normal injection of 1 s by MEKC (Fig. 5a) and correction by the dilution factor (in this case 10 000 for PQ and DQ and 2000 for DF). The enhancement factors obtained were 5000 for DF and 21 000 for PQ and DQ in terms of peak height.

3.4. Quality parameters

Figures of merit using both sweeping–MEKC and CSEI–sweeping–MEKC methods under optimal conditions were calculated and the results are given in Table 1. The LODs based on a signal-to-noise ratio of 3:1 and expressed as μg l^{-1} of quaternary ammonium ion were about 10 when sweeping–MEKC was used. These LODs are similar to those obtained in a previous work using stacking with sample matrix removal as an on-line concentration procedure [35] and are 30–80 times lower than those obtained in a previous work using conventional capillary zone electrophoresis with hydrodynamic injection [44]. When CSEI–sweeping–MEKC was used, LODs lower than 1 μg l^{-1} were obtained. In this case, the LODs are similar, and slightly lower for PQ and DQ, than those obtained in a previous work using the combination of two preconcentration procedures, solid-phase extraction (SPE) and stacking with sample matrix removal [33]. However, the latter technique suffers from longer analysis time and laborious sample handling. For PQ and DQ, these LODs are below the maximum permitted levels established by both the EPA [6,7] and the European Union [8]. It should be noted that the relative sensitivity enhancement achieved with both sweeping methods, obtained by comparing the limits of detection with those of conventional MEKC, were between 250- and 500-fold enhancement for sweeping–MEKC and between 3000 and 50 000 for CSEI–sweeping–MEKC.

To obtain run-to-run repeatability information, a total of six replicates (~100 μg l^{-1} for sweeping–MEKC, and ~10 μg l^{-1} for PQ, DQ and EV and ~50 μg l^{-1} for DF for CSEI–sweeping–MEKC) determinations were performed under optimal conditions in the same day. The day-to-day reproducibility was calculated by performing six replicate determinations of the same standard solution in 3 days (two replicates each day). The relative standard deviations (RSDs) obtained by sweeping–MEKC were lower than 4.7% for migration time and lower than 7.9% for concentration. CSEI–sweeping–MEKC shows

Table 1
Quality parameters

Parameter	PQ[a]		DQ[b]		DF[a]	
	Sweeping	CSEI–sweeping	Sweeping	CSEI–sweeping	Sweeping	CSEI–sweeping
Limit of detection (μg l^{-1})	10.2	0.075	10.1	0.1	13.0	1.0
Sensitivity enhancement (SEc)[c]	250	35 000	500	51 000	250	3000
Run-to-run repeatability, RSD (%) ($n=6$)						
Migration time	4.7	6.6	4.3	6.1	2.7	4.2
Concentration[d]	5.1	5.9	5.6	5.4	5.7	7.5
Day-to-day reproducibility, RSD (%) ($n=2\times3$)						
Migration time	4.3	14.2	4.1	12.4	3.7	7.2
Concentration[d]	7.1	8.9	5.7	20.8	7.9	22.6
Working range (μg l^{-1})	20.4–306.1	0.5–25.5	20.3–304.9	0.5–25.4	26.1–391.4	6.5–130.5
Linearity (r^2)	0.9997	0.9994	0.9997	0.9995	0.9985	0.9995

[a] λ: 255 nm.
[b] λ: 220 nm.
[c] SEc = LOD(MEKC)/LOD (sweeping–MEKC or CSEI–sweeping–MEKC).
[d] Obtained by external calibration using EV as internal standard.

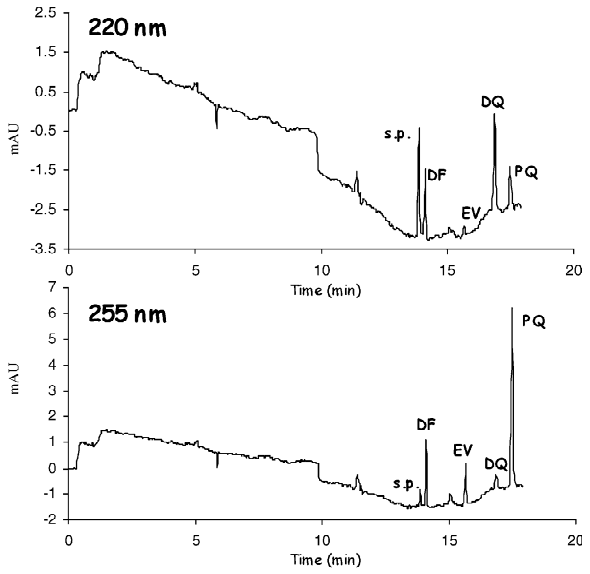

Fig. 6. Electropherogram of tap water analysed by CSEI–sweeping–MEKC. Sample concentration: 10 μg l^{-1} PQ, DQ and EV, 50 μg l^{-1} DF. Other conditions as in Fig. 5b.

higher RSDs due to the poor reproducibility of the electrokinetic injection [47]. Calibration curves based on the peak height ratio (compound/internal standard) for PQ, DQ and DF at the working ranges indicated in Table 1 were obtained and good linearity, with correlation coefficients (r^2) higher than 0.998, was observed.

3.5. Application

To demonstrate how the CSEI–sweeping–MEKC method can be applied for routine analysis of real samples, quat-spiked tap water samples were analysed. Fig. 6 shows the electropherograms obtained when Japanese tap water (Harima Science Garden) spiked at 10 μg l^{-1} for PQ, DQ and EV and at 50 μg l^{-1} for DF was injected using the optimized method. The limits of detection, expressed as μg l^{-1} of quaternary ammonium herbicides and based on a signal-to-noise ratio of 3:1, are given in Table 2 and were 0.5 for PQ, 1.0 for DQ and 3.3 for DF. These limits of detection are higher than those obtained when standards in purified water were used, due to the relatively high salinity of tap water (conductivity: 152.4 μS cm^{-1}) that produced a low field enhancement when the electrokinetic injection was used. The values are similar to those obtained in a previous work using SPE–stacking [33] for tap water. Moreover, for PQ and DQ the LODs obtained are sufficient for the analysis of these compounds in drinking water at the levels established by the EPA. The method was used to quantify a Japanese tap water spiked at 1.0 and 5.0 μg l^{-1} for PQ and DQ, respectively (values below the EPA levels) and at 6.5 μg l^{-1} for DF. Quantitation was performed by standard addition ($n=3$) and the results in terms of concentration and standard deviations are given in Table 2 showing that good accuracy and precision were obtained using this method.

4. Conclusions

Sensitive methodologies based on sweeping–MEKC and CSEI–sweeping–MEKC for the analysis of quats have been developed. Enhancement of lower than 240- and 50 000-fold was obtained when sweeping–MEKC and CSEI–sweeping–MEKC were

Table 2
Application: analysis of spiked tap water

	LODs (μg l^{-1})	LODs SPE–stacking (μg l^{-1})[c]	Spiked value (μg l^{-1})	Found value (μg l^{-1})[d]
PQ[a]	0.5	0.4	1.0	1.0±0.2
DQ[b]	1.0	2.2	5.0	4.9±0.5
DF[a]	3.3	1.1	6.5	6.3±0.2

[a] λ: 255 nm.
[b] λ: 220 nm.
[c] Ref. [33].
[d] Obtained by standard addition ($n=3$) using EV as internal standard.

applied, respectively, providing LODs down to 0.075–1.0 μg l^{-1} for standards in purified water when CSEI–sweeping–MEKC was used. Acceptable run-to-run and day-to-day reproducibilities were obtained for both on-line concentration methods. The results of this study demonstrated that CSEI–sweeping–MEKC may be used for the determination of trace amounts of the quaternary ammonium herbicides in drinking water. LODs between 0.5 and 1.0 μg l^{-1} were obtained for paraquat and diquat in drinking water allowing the analysis of these compounds at the levels established by the EPA.

Acknowledgements

O. Núñez is grateful to the Generalitat de Catalunya (Spain) for a grant (Beca predoctoral per a la formació de personal investigador, FI) and for the financial support of a stay in Japan.

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