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Sample stacking with matrix removal for the determination of paraquat, diquat and difenzoquat in water by capillary electrophoresis☆

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Abstract

Conditions for the simultaneous determination of paraquat, diquat and difenzoquat by capillary zone electrophoresis using a stacking technique in a chemically modified capillary have been established. To apply the stacking method with sample matrix removal for the analysis of cations, an anodic electroosmotic flow is mandatory. For quats, 50 m*M* acetic acid–ammonium acetate (pH 4.0) with 5% (v/v) methanol as electrophoretic buffer and the addition of 0.8 m*M* cetyltrimethylammonium bromide as wall capillary organic modifier was proposed. Field polarity reversal time was optimised for several sample matrices. Detection was carried out at 220 and 255 nm. Detection limits, based on a signal-to-noise ratio of 3:1, were lower than 15 $\mu g\ l^{-1}$ for standards in Milli-Q water and two to ten times higher for drinking water samples. Run-to-run and day-to-day reproducibility have been established. The method was successfully applied to the determination of the three herbicides in spiked drinking water.

Keywords: Water analysis; Sample stacking; Sample handling; Quaternary ammonium herbicides; Pesticides

1. Introduction

Some quaternary ammonium herbicides, named ''quats'', have been widely used in agricultural applications. Paraquat (1,1′-dimethyl-4,4′-bipyridylium ion, PQ) and diquat (1,1′-ethylene-2,2′-bipyridylium ion, DQ) are used mainly as non-selective herbicides, and difenzoquat (1,2-dimethyl-3,5-diphenylpyrazolium ion, DF) as a selective herbicide [1]. The herbicide structures are shown in Fig. 1. Quats are toxic to man and have been classified as

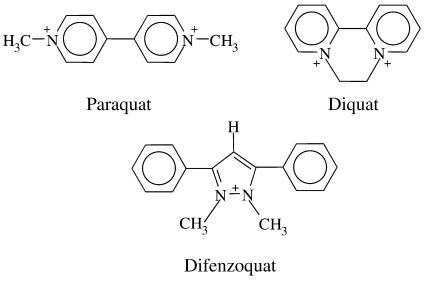

Fig. 1. Quaternary ammonium herbicide structures.

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moderately hazardous by the World Health Organisation [2] and some have been included on ''priority'' lists and are regulated in drinking water in Mediterranean countries [3]. For drinking water, the US Environmental Protection Agency has established a maximum contamination level (MCL) of 20 μg l^{-1} for DQ and a goal of 3 μg l^{-1} for PQ [4,5].

The determination of these compounds is difficult due to their cationic character. Nevertheless, their analysis in several matrices has been reported using spectrophotometry [6,7], spectrofluorimetry [8], ion-selective electrodes [9,10], enzyme-linked immunosorbent assay (ELISA) [11,12], gas chromatography [13,14] and gas chromatography–mass spectrometry [15]. Ion-pair high-performance liquid chromatography with direct UV detection has also been used for the simultaneous determination of paraquat, diquat and difenzoquat [16,17]. In order to improve selectivity and sensitivity, liquid chromatography coupled to mass spectrometry (LC–MS) has been used [18–20]. Capillary electrophoresis (CE) has been applied to the determination of quaternary ammonium herbicides in water [16,21–23] and other matrices such serum [24,25], soil and urine [24]. Moreover, CE–MS has also been used for the determination of these compounds in water samples [26–28].

Capillary electrophoresis using on-column UV detection is hindered by the short optical path defined by the column diameter. Although the mass limit in CE can be very low because of the small volume, the detection limit is usually ~10^{-6} *M*, which is several orders of magnitude higher than the detection limits for LC, and so preconcentration techniques are needed. Off-line preconcentration procedures contribute to reaching low detection limits but involve long analysis times and laborious sample handling. Several techniques for on-column preconcentration have been reported. Isotachophoresis (ITP) [29,30] has been used as on-line water sample pre-treatment for the determination of PQ and DQ by CE [31]. The major disadvantage of ITP is the need to use several types of support buffers in a single capillary column, a process known as discontinuous electrophoresis. Other on-column preconcentration techniques involve several sample stacking procedures [32–34], the simplest of which consists of the injection of large volumes of sample dissolved in a lower conductivity buffer matrix than those used for CE separation. In these conditions, the charged species are focused in a sharp sample band, and the detection limit is decreased ten-fold. However, the amount of sample which can be loaded into the capillary in conventional sample stacking is rather limited because of disturbances caused by the low-concentration sample buffer [33,35]. To stack an extremely long sample plug while retaining high resolution, the sample buffer must be removed after stacking is completed in order to eliminate the non-uniform distribution of both the field strength and electroosmotic velocity.

One method of pumping out the sample matrix is to use the electroosmotic flow (EOF) while the sample stacking is in progress. The most usual procedure to do this involves field polarity reversal when the capillary has been filled with a large volume of sample of lower conductivity than those used for CE separation. To apply this method to a cationic analyte, which has a positive electrophoretic mobility with respect to the electroosmotic flow, the direction must be reversed, for instance by adding a cationic surfactant.

In this work, we used the stacking concentration method with sample matrix removal in a single, continuous support buffer, to enhance quaternary ammonium herbicide detection. Cetyltrimethylammonium bromide (CTAB) was used as wall capillary modifier to make the charge on the silica wall positive and thus reverse the electroosmotic flow. Quality parameters were obtained and the method was applied to the analysis of tap water and mineral water samples.

2. Experimental

2.1. Instrumentation

A Beckman P/ACE 5500 capillary electrophoresis system (Fullerton, CA, USA) equipped with diode array detection was used. This system was modified to control the reversal of the electrode polarity. Electrophoretic separations were carried out using uncoated fused-silica capillaries (Supelco, Bellefonte, PA, USA) with a total length of 57 cm, separation length of 50 cm, and internal diameter of

50 μm. New capillaries were pre-treated using 0.1 *M* hydrochloric acid for 15 min, ultrapure water for 15 min, 1 *M* sodium hydroxide for 30 min, and finally rinsed with ultrapure water for 30 min. The capillary was conditioned daily using 1 *M* sodium hydroxide for 15 min, then rinsed with ultrapure water for 15 min and finally with the running buffer for 60 min before the first run. Conditioning was carried out between runs using a running buffer for 2 min. Acetic acid–ammonium acetate 50 m*M* buffer solution at pH 4.0 containing 5% methanol and 0.8 m*M* CTAB as wall capillary modifier was used as running buffer. Carrier electrolyte was filtered through a 0.45-μm membrane filter, and degassed before use. The temperature was held at 25°C and direct UV detection was performed at 255 and 220 nm. Samples were loaded by pressure injection at 140 kPa and an injection time of 0.25 min. For sample matrix removal, +20 kV were applied and the separation was performed by reversing the polarity at the same potential. Electrophoretic data were processed with Beckman P/ACE Station software version 1.0.

2.2. Chemicals

The reagents, all of analytical grade, were obtained from the following sources: DQ (97%) and DF (98%) were purchased from Chemservice (West Chester, PA, USA), and PQ from Sigma (St. Louis, MO, USA). Ethyl viologen (EV) (Aldrich, Milwaukee, WI, USA) and hepthyl viologen (HV) (TCI, Tokyo, Japan) were used as internal standards. HPLC-gradient grade methanol, acetic acid (100%), sodium hydroxide, hydrochloric acid (25%) and CTAB were purchased from Merck (Darmstadt, Germany), ammonium acetate from Fluka (Buchs, Switzerland) and phenol from Carlo Erba (Milan, Italy). Water was purified using an Elix 3 coupled to a Milli-Q system (Millipore, Bedford, MA, USA).

Stock standard solutions of individual quats and internal standards (1 mg ml^{-1}) were prepared in Milli-Q water and stored in plastic vials to prevent adsorption. Working solutions were obtained by dilution with 0.8 m*M* CTAB aqueous solution, and were filtered through a 0.45-μm nylon filter. Buffers were prepared from an aqueous solution of acetic acid 400 m*M* and the pH was adjusted with ammonium acetate or sodium acetate (400 m*M*). This buffer was diluted with Milli-Q water to 50 m*M* after the addition of CTAB and methanol to obtain the final carrier electrolyte.

2.3. Stacking procedure

To reverse the electroosmotic flow a cationic surfactant, CTAB, at a concentration of 0.8 m*M* was added to the carrier electrolyte and the samples. CTAB concentration was always under its critical micelle concentration (0.92 m*M* under aqueous conditions) to avoid micelle formation. Phenol was used as EOF marker. The determination of quats in tap water (883 $\mu\Omega^{-1}$ cm^{-1}) and mineral water (474 $\mu\Omega^{-1}$ cm^{-1}) required dilution 1:4 with Milli-Q water (1.6 $\mu\Omega^{-1}$ cm^{-1}) prior to analysis.

The stacking procedure involved several steps. The capillary was first filled with the carrier electrolyte and then a long plug of sample was introduced hydrodynamically by pressure (140 kPa) during 0.25 min. A high voltage (+20 kV) was then applied and the electric current was monitored to indicate when the sample matrix was almost removed from the capillary. After injection, the current decreased due to the high resistivity caused by the presence of the sample. As the sample matrix was pushed out of the capillary, the current increased. When the current was 95% of the original carrier electrolyte value the voltage was turned off and the electrodes were switched to the separation configuration.

3. Results and discussion

3.1. Stacking and CE separation conditions

To apply the stacking technique with sample matrix removal to the determination of quats, CTAB was used as surfactant to reverse the EOF towards the anode. At low pH (below pH 4.0) there was a marked number of dissociated silanol groups (Si–O^-) in the internal capillary wall. Due to association of CTAB long hydrocarbon chains, a reversal in the zeta potential of the double layer at the capillary internal surface occurred at a certain critical concentration of surfactant. To evaluate the effect of the

counter ion, two buffers were studied, acetic acid–ammonium acetate and acetic acid–sodium acetate, both at pH 4.0. The CTAB concentration and carrier electrolyte concentration were optimised for each buffer to obtain an electrophoretic flow high enough to elute the quaternary ammonium herbicides, which have electrophoretic mobilities in opposite directions. For this purpose, a buffer concentration in the range 50–240 m*M* and CTAB concentration from 0.3 to 0.8 m*M* were studied.

Fig. 2 shows the variation of EOF versus buffer concentration at several CTAB concentrations. When using 0.8 m*M* CTAB, a decrease in buffer concentration produced an increase in the EOF, due to the effect of ionic strength. When ammonium buffer was used at low CTAB concentrations (0.5 and 0.3 m*M*), a decrease in the EOF was observed as buffer concentration decreased. This behavior was specially marked at concentration levels below 100 m*M*. In contrast, an increase was always observed for sodium buffer. This may be due to the distinct adsorption of the buffer counter ion on the capillary wall. The adsorption of the ammonium ion was stronger than that of the sodium, thus competition with the cetyltrimethylammonium ion for the capillary surface occurred and, as a result, the capillary wall was not totally coated with the surfactant. The negative charge density within the diffused layer decreased and lower EOF values for the ammonium ion were observed. Therefore, to obtain a high EOF, a low ammonium buffer (50 m*M*) and high CTAB concentration (0.8 m*M*) were used for the remainder of this study.

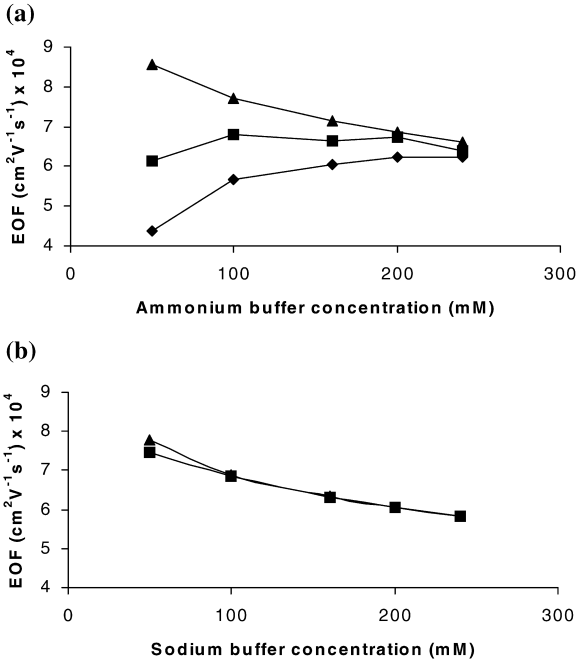

Fig. 2. Effect of buffer and surfactant concentration on the electroosmotic flow. (a) Acetic acid–ammonium acetate (pH 4.0). (b) Acetic acid–sodium acetate (pH 4.0). ♦ 0.3 m*M* CTAB, ■ 0.5 m*M* CTAB, ▲ 0.8 m*M* CTAB.

Fig. 3 shows the electropherograms obtained in the above mentioned conditions, at two wavelengths (220 and 255 nm). The three cationic herbicides and the two internal standards appeared after the electroosmotic flow marker and the bromide counteranion of some quaternary ammonium herbicide standards migrated before the EOF marker.

To achieve an efficient stacking effect the field polarity reversal time must be established. This time was affected by electrolyte composition, capillary conditioning and sample matrix. Therefore the reversal time must be controlled for each buffer and sample. To study the influence of this parameter on the separation, standard solutions of the three her-

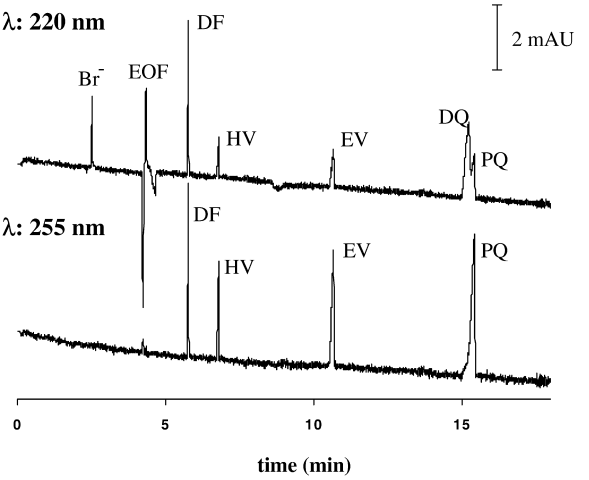

Fig. 3. Electropherograms, at two wavelengths, of a standard solution of PQ (21.4 mg l^{-1}), DQ (20.7 mg l^{-1}) and DF (20.4 mg l^{-1}) and the internal standards EV (21.1 mg l^{-1}) and HV (20.4 mg l^{-1}) in Milli-Q water. Carrier electrolyte: acetic acid–ammonium acetate 50 m*M* (pH 4.0), 0.8 m*M* CTAB. Hydrodynamic injection, 10 s. Applied potential, +20 kV. Phenol (20.8 mg l^{-1}) was used as an EOF marker.

bicides and the two internal standards in Milli-Q water were used. The capillary current was monitored to determine the reversal time. Before injection, the current of a capillary completely filled with electrophoretic buffer was measured applying +20 kV. Then, the standard solution containing 0.8 m*M* CTAB was hydrodynamically injected into the capillary (0.25 min, 140 kPa) and a voltage of +20 kV was applied. After the injection, the current level decreased due to the increase in resistivity caused by the ionic concentration of the sample. As the sample matrix was pushed out of the capillary, the current increased until the initial value. The polarity was reversed when the electric current was 95% of the initial value, and 2.3 min were needed.

When the reversal time was too high, DF was not detected because it was completely pushed out of the capillary with the sample matrix due to its low electrophoretic mobility. In contrast, when it was too low, a poor resolution between PQ and DQ was observed due to the long plug of sample in the capillary. The reversal time was critical due to the distinct electrophoretic behavior of the double-charged herbicides, PQ and DQ, which have high electrophoretic mobilities, and the monocharged herbicide DF, with low electrophoretic mobility. The difference in the migration time for these herbicides was above 10 min (Fig. 3).

Fig. 4a shows that the herbicides can be detected by applying the stacking method in a short analysis time. Although a system peak appeared (9.7 min), the electrophoretic separation was not affected. Nevertheless the EV peak, which migrated near to the system peak, showed a disturbance that prevented the use of this compound as internal standard. To improve the resolution between PQ and DQ, small amounts of methanol (0.5–8%) were added to the electrophoretic buffer. This addition increased the resolution between PQ and DQ but the analysis time was also augmented. As a compromise, 5% methanol was used (Fig. 4b) because a baseline resolution between both herbicides in a relatively short analysis time was obtained. Consequently, 50 m*M* acetic acid–ammonium acetate at pH 4.0 with 5% methanol and 0.8 m*M* CTAB was proposed as the optimal electrophoretic carrier electrolyte for the analysis of these compounds.

3.2. Quality parameters

Quality parameters using stacking with sample matrix removal under optimal conditions are given in Table 1. The detection limits, expressed as $\mu g\ l^{-1}$ of quaternary ammonium ion, are based on a signal-to-noise ratio of 3:1 and were lower than 15 $\mu g\ l^{-1}$. These detection limits are 30–80 times lower than

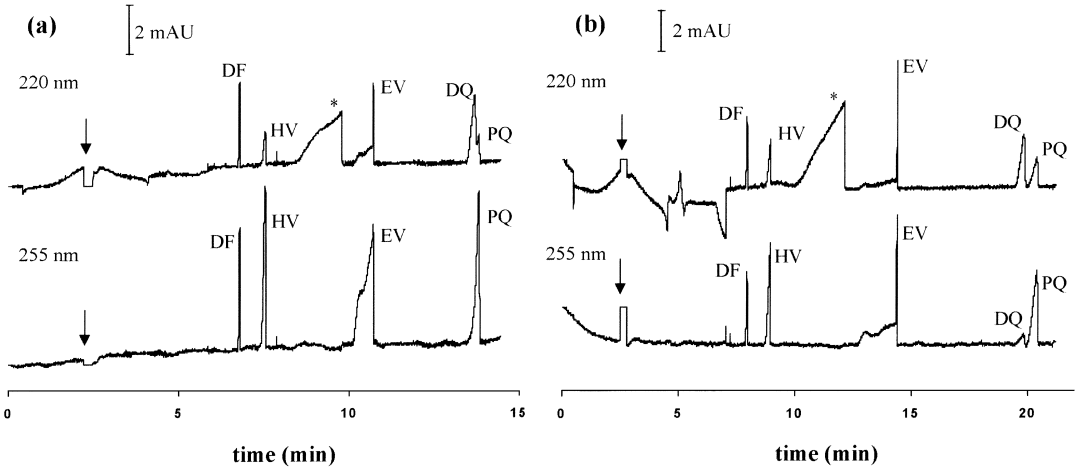

Fig. 4. Electropherogram of a standard solution of PQ (0.2 mg l^{-1}), DQ (0.2 mg l^{-1}) and DF (0.2 mg l^{-1}) and the internal standards EV (0.8 mg l^{-1}) and HV (0.8 mg l^{-1}) in Milli-Q water. Hydrodynamic injection, 0.25 min (137.9 kPa). Applied potential, +20 kV (sample matrix removal), −20 kV (electrophoretic separation). Carrier electrolyte: (a) acetic acid–ammonium acetate (pH 4.0), 0.8 m*M* CTAB. (b) as (a) with 5% MeOH. *, system peak. The arrow shows the reversal time.

Table 1
Quality parameters

Parameter		PQ[a]			DQ[b]			DF[a]		
		Milli-Q[c] water	Tap water	Mineral water	Milli-Q[c] water	Tap water	Mineral water	Milli-Q[c] water	Tap water	Mineral water
LOD ($\mu g\ l^{-1}$)		10	48	18	11	64	25	15	154	62
LOD hydrodynamic injection ($\mu g\ l^{-1}$)[d]		829	820	–	960	950	–	435	425	–
LOD electrokinetic injection ($\mu g\ l^{-1}$)[e]		2.9	21	–	2.3	18	–	3.9	31	–
Migration time										
Run-to-run reproducibility, RSD (%) ($n=5$)		1.3	0.8	0.2	1.2	0.1	0.2	0.3	0.1	0.1
Day-to-day reproducibility, RSD (%) ($n=15$)		8.8	–	–	8.4	–	–	1.6	–	–
Concentration (spiked sample)										
Run-to-run reproducibility,	(1)	3.7	2.5	3.3	6.8	3.6	2.4	2.3	5.4	4.6
RSD (%) ($n=5$)[f]	(2)	–	4.6	2.8	–	3.5	3.2	–	3.7	1.8
Day-to-day reproducibility, RSD (%) ($n=15$)		13.1	–	–	15.4	–	–	2.4	–	–
Linearity (30–850 $\mu g\ l^{-1}$)										
Correlation coefficients[f]	(1)	0.992	0.999	0.999	0.994	0.999	0.999	0.999	0.999	0.999
	(2)	–	0.999	0.999	–	0.999	0.996	–	0.994	0.997

[a] λ: 255 nm.
[b] λ: 220 nm.
[c] Using HV as internal standard.
[d] Ref. [36].
[e] Ref. [16].
[f] (1) External calibration; (2) standard addition.

those obtained in a previous study using hydrodynamic injection but are slightly worse than those obtained using electrokinetic injection [36] although the reproducibility is improved.

A total of five replicate determinations of a standard solution of PQ, DQ and DF (0.4 mg l^{-1}) were carried out under optimum conditions to determine run-to-run reproducibility. Relative standard deviations (RSD) of migration time (0.3–1.3%) and concentration determined by external calibration (2.3–6.8%) were obtained. To determine day-to-day reproducibility, five replicate analyses of the same standard solution were made on 3 days ($n=15$). RSDs in the range 1.6–8.8% for migration time and 2.4–15.4% for concentration were obtained. DF showed the best results probably due to the lower analysis time and the internal standard used (HV), that migrated close to it. Calibration curves based on the peak area ratio (compound/internal standard) for paraquat, diquat and difenzoquat at concentrations between 30 and 850 $\mu g\ l^{-1}$ were obtained and good linearity was observed.

3.3. Application

To show the applicability of the method for the routine analysis of real samples, spiked tap water from Barcelona and mineral water were used. When applying the stacking method with sample matrix removal to the analysis of these samples, the signal obtained was lower and the resolution between PQ and DQ was worse than those obtained for standards, probably due to the relatively high conductivity of the samples, tap water (883 $\mu\Omega^{-1}\ cm^{-1}$) mineral water (474 $\mu\Omega^{-1}\ cm^{-1}$). Since the stacking method requires a considerable difference between the sample region and the carrier electrolyte conductivity,

the salinity of the sample must be taken into account. Therefore, a dilution of the sample with Milli-Q water was necessary to increase the efficiency of the preconcentration step.

In the electropherograms of the spiked sample without dilution and diluted 1:1 (Fig. 5a2 and a3) a poor resolution between PQ and DQ was obtained, and DF did not appear. When the dilution factor increased, the resolution improved and DF was detected (Fig. 5a4 and a5). These electropherograms have been acquired at 200 nm, because at this wavelength the three herbicides can be detected simultaneously. The increase in resolution between PQ and DQ can be explained by the stacking effect, which is very pronounced with samples of low conductivity (Fig. 5b). Nevertheless, dilution produced a decrease in the peak areas. In contrast, DF was detected when the sample was sufficiently diluted, because the matrix effect was prevented as the high efficiency of the stacking with sample matrix removal increased. As a compromise between response and PQ/DQ resolution, a dilution of 1:4 was proposed.

Quality parameters using stacking with sample matrix removal under optimal conditions for water samples are given in Table 1. The detection limits, expressed as $\mu g\ l^{-1}$ of quaternary ammonium ion, are based on a signal-to-noise ratio of 3:1 and were slightly higher than those obtained for water samples using electrokinetic injection [16]. The values for mineral water were three times lower than for tap water due to its lower salinity. These detection limits were higher than those obtained for Milli-Q water spiked samples, thus showing the contributory effect of sample matrix. Nevertheless these values are higher than those established by the US Environmen-

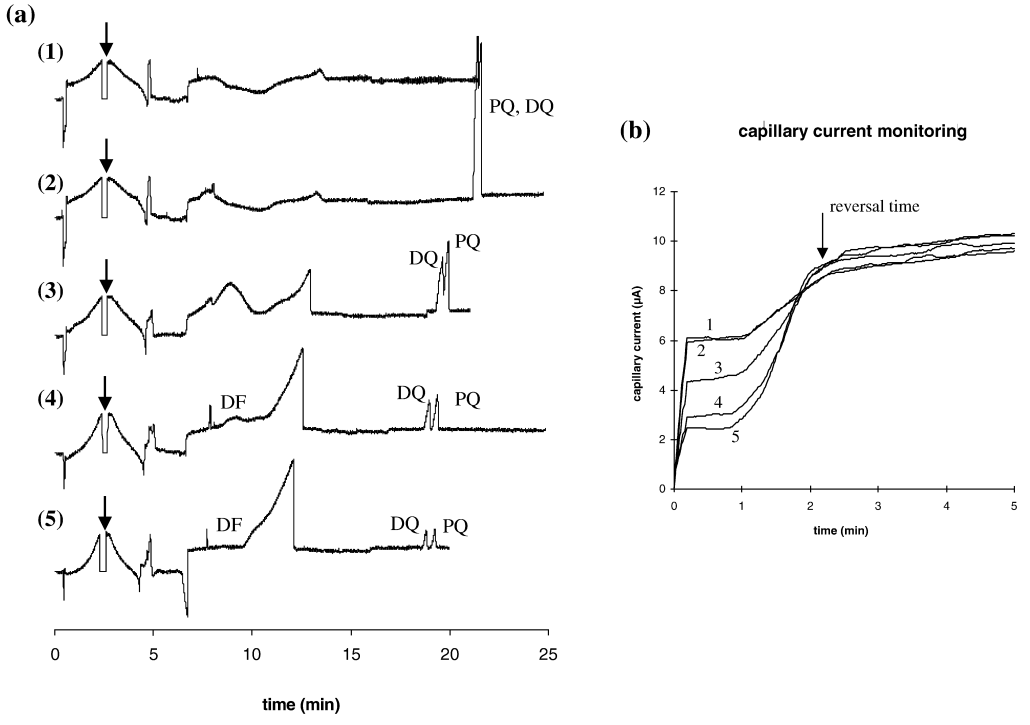

Fig. 5. (a) Electropherogram of tap water and (b) capillary current monitoring. (1) Non-spiked water; (2) spiked water at 1000 $\mu g\ l^{-1}$; (3) spiked water diluted 1:1; (4) spiked water diluted 1:4; (5) spiked water diluted 1:9. Running buffer: acetic acid–ammonium acetate (pH 4.0), 0.8 m*M* CTAB, 5% MeOH. λ: 200 nm.

tal Protection Agency [4,5] for drinking water, so the method can only be applied for relatively highly contaminated waters.

A total of five replicate determinations of spiked tap water and mineral water at a level of 1 mg l^{-1} were carried out under optimum conditions to determine run-to-run reproducibility. In these samples neither EV nor HV could be used as internal standards due to disturbances produced by the system peak. Therefore quantification was carried out using both external calibration with standard solutions of PQ, DQ and DF in Milli-Q water in the range from 30 to 850 μg l^{-1}, and standard addition. RSDs based on concentration were from 2.5 to 5.4%. Both quantitation methods can be applied to the analysis of drinking water samples.

4. Conclusions

In this paper the suitability of stacking using a cationic surfactant to reverse EOF for the separation of cations with a wide range of mobilities has been verified. CTAB in a buffer solution with methanol was needed to reverse the flow and obtain a convenient separation of PQ and DQ. Reversal time is critical and must be established for each sample. Sample matrix contributes to the performance of the procedure and a sample dilution is mandatory to improve both resolution and sensitivity. Sample stacking with EOF reversal has proven to be a convenient method for the analysis of paraquat, diquat and difenzoquat in highly contaminated water samples. Actually additional studies are being carried out to improve the limits of detections by combining stacking procedure with other preconcentration methods.

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