Comparison of capillary electrophoresis and reversed-phase ion-pair high-performance liquid chromatography for the determination of paraquat, diquat and difenzoquat ☆

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Abstract

The conditions were established for the simultaneous determination of paraquat, diquat and difenzoquat by high performance liquid chromatography (HPLC) and capillary electrophoresis (CE). For the HPLC separation, the use of a reversed phase, heptanesulphonate as ion-pairing agent and an aqueous–acetonitrile mobile phase with stepwise elution from 93:7 to 70:30 was adopted. Acetic acid–sodium acetate (pH 4.0) with 100 mM sodium chloride as running buffer and electrokinetic injection were used in CE. The figures of merit were calculated and the two techniques were compared. Detection limits (signal-to-noise ratio = 3:1) ranged from 2.9 to 5.5 μg l^{-1} and were similar for both techniques when standards were dissolved in water, but when CE was used the response was greatly affected by the nature of the sample matrix. The run-to-run and day-to-day reproducibilities and the analysis times were similar for both techniques. However, CE did not require preconditioning and a long stabilization period was needed in ion-pair HPLC. The methods were applied to the determination of the herbicides in crop waters.

1. Introduction

Since the discovery of their herbicidal potential in the mid-1950s, some quaternary ammonium salts, named "quats", have been used extensively for the control and management of terrestrial and aquatic vegetation. The bipyridylium herbicides 1,1'-dimethyl-4,4'-bipyridinium salts (paraquat) and 1,1'-ethylene-2,2'-bipyridyldiylium salts (diquat) are effective contact desiccants widely used in preharvest

desiccation of various crops, in pasture renovation and for post-emergent non-selective weed control [1,2]. The pyrazolium monocation 1,2-dimethyl-3,5-diphenylpyrazolium (difenzoquat) is a more selective herbicide used for the post-emergence control of wild oats in wheat and barley [3,4].

Paraquat and diquat are pesticides of major economic importance, they are toxic to man and have been classified as moderately hazardous. Their use has been restricted and in some countries maximum limits in drinking water between 0.3 and 200 μg l^{-1} have been established [5].

Analyses for these compounds in water [6–8], agricultural products [9–11], blood [12,13], urine

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[14,15] and biological tissues [16] have been published. The methods used include spectrophotometry [17–19], ion-selective electrodes [20,21], spectrophotometric sensors [22], gas chromatography [7,23,24], gas chromatography-mass spectrometry [7,25] and liquid chromatography-mass spectrometry [26]. Most of these methods suffer from deficiencies. Spectrophotometric methods involve extensive sample treatment and are time consuming, ISE methods are suitable for diquat but not for paraquat [20] and gas chromatographic methods are sensitive but require prior hydrogenation or pyrolysis and give poor reproducibility at low concentration levels [7]. Ion-pair high-performance liquid chromatography [15,16,27] and capillary electrophoresis [8] have also been described and seem to give better results, but no procedure for the simultaneous determination of the three compounds, paraquat, diquat and difenzoquat, in a single sample has been described. Recently, we proposed a method for the determination of these compounds by capillary electrophoresis [28]; the method allows their separation and determination with good resolution and reproducibility and low detection limits.

In this paper, conditions for the simultaneous determination of these three compounds by ion-pair reversed-phase liquid chromatography were established and the quality parameters were calculated. A comparison between capillary electrophoresis and liquid chromatography for the determination of these compounds was performed and advantages and disadvantages were compared.

2. Experimental

2.1. Chemicals

Reagents used for the preparation of buffer solutions and mobile phases were analytical-reagent grade acetic acid, sodium hydroxide and triethylamine (TEA) from Merck (Darmstadt, Germany), analytical-reagent grade sodium chloride and phosphoric acid from Carlo Erba (Milan, Italy), sodium heptanesulphonate (SHS) from MTM Lancaster Research Chemicals

(Strasbourg, France) and HPLC-grade acetonitrile (ACN) from Merck. Paraquat (PQ) (99%) was purchased from Riedel-de Haën (Seelze, Germany) and diquat (DQ) (97%) and difenzoquat (DF) (98%) from Chemservice (West Chester, PA, USA). Water was purified using a Culligan (Barcelona, Spain) system. All solutions were passed through a 0.45-μm nylon filter before use.

2.2. Apparatus and conditions

HPLC was carried out on a Hewlett-Packard (Waldbronn, Germany) Series 1050 liquid chromatograph with a quaternary pump, a variable-wavelength detector and an automatic injector. Data were collected and integrated by a Hewlett-Packard Vectra QS/16S computer. A Merck LiChrospher 100 RP-18 (5 μm) column (12.5 cm × 4 mm I.D.) was used at room temperature. An aqueous solution of 10 mM sodium heptanesulphonate, 100 mM phosphoric acid and 100 mM triethylamine mixed with acetonitrile in the ratio 93:7 (v/v) (phase A) and 60:40 (v/v) (phase B) were used as mobile phases; the flow-rate was 1.3 ml min^{-1}. Both solutions were passed through a 0.45-μm filter (Millipore, Bedford, MA, USA). The mobile phase was degassed for 15 min with helium. The chromatographic system was conditioned for 12 h with mobile phase A to obtain reproducible results. The sample volume was 100 μl. The detection wavelength was at 257 nm for PQ and DF and 310 nm for DQ.

Capillary electrophoretic analysis was performed with an Applied Biosystems (CA, USA) Model 270A capillary electrophoresis system with spectrophotometric detection. Electrophoretic data were processed with a Merck–Hitachi Model 2500 integrator. A fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA), 50 μm I.D. and 72 cm length with a separation length of 50 cm, was used. The temperature was held at 30°C and the applied potential was +15 kV. UV detection was performed at 205 nm. Electrokinetic (10 s) and hydrodynamic (3 s) injection modes were used. An acetic acid–sodium acetate buffer solution at pH 4.0 [29] containing 100 mM sodium chloride was used as the

running buffer. Initial equilibration was performed for 2 h using the running buffer. After each run the capillary was washed with 0.01 M sodium hydroxide for 2 min and equilibrated with running buffer for 5 min. The conductivity was measured with a Model CDM83 conductivity meter from Radiometer (Copenhagen, Denmark).

3. Results and discussion

3.1. High-performance liquid chromatography

Ion-pair liquid chromatography with UV detection has been proposed [15,16,27] for the determination of quaternary ammonium compounds with sodium salts of alkylsulphonates as ion-pairing agents. A drawback to using a single ion-pair LC method for the simultaneous determination of PQ, DQ and DF is the different conditions required for the elution of each compound. Mobile phases with high elution strengths [aqueous buffer solution–acetonitrile (70:30)] are proposed for DF, whereas lower elution strengths are used [aqueous buffer solution–acetonitrile (90:10)] when PQ and DQ are determined [27].

The effect of ACN on the separation of PQ and DQ was studied. A resolution between PQ and DQ higher than 1.5 was obtained with ACN concentrations lower than 14%, but under these conditions DF was not eluted. Therefore, an isocratic elution mode was not possible for the simultaneous determination of the three compounds. In order to achieve a single method of analysis, several gradients were investigated using two mobile phases. Generally, low concentrations of ion-pairing agent, about 10 mM, were used. When a gradient was used a wide DF peak on the tail of a large disturbance in the baseline was observed. A narrow and more reproducible DF peak was obtained by increasing the concentration of ion-pairing agent in the mobile phase to 40 mM and performing the gradient with stepwise elution from 7% to 30% ACN, as can be seen in Fig. 1, where the chromatograms using a 6-min gradient and step-

wise elution are compared. Detection was performed at two wavelengths to improve the sensitivity of the method.

3.2. Capillary electrophoresis

Capillary electrophoresis has proved to be a suitable separation technique for the simultaneous determination of PQ, DQ and DF [28]. Fig. 2 shows a typical electropherogram of these quaternary ammonium compounds obtained in a single run using an acetic acid–sodium acetate buffer solution at pH 4.0 with 100 mM sodium chloride as running buffer. In environmental analyses for these compounds it is often necessary to monitor concentrations below 200 ng ml^{-1}, and such levels can be achieved using capillary electrophoresis. In this technique, the detection limits and precision depend on the injection mode and, moreover, operating procedures and sample matrix effects such as sample volume and electrolyte concentration must be considered.

In this work, both hydrodynamic (by vacuum) and electrokinetic injection modes were studied. The sensitivity, in terms of response factors, was found to be strongly influenced by the injection mode. Table 1 gives normalized response factors in both water and running buffer solutions for each injection mode. Area response factors for each compound using equal concentration and injection time bases were calculated, and they were related to the lowest DQ value to obtain normalized response factors. Higher sensitivities were found using the electrokinetic injection mode provided that the analytes were dissolved in water. Moreover, using this injection mode, the detector responses decreased by a factor of 8–35 when the analytes were dissolved in the running buffer solution. Electroinjection of samples with low conductivity (e.g., analytes dissolved in pure water) into a column filled with a high-conductivity buffer solution results in an electric field at the injection point that is much stronger than the electric field in the column and, therefore, a field-amplified sample injection occurs [30]. Thus, for the quaternary ammonium ions a very high electrophoretic velocity was obtained at the injection point. The total amount
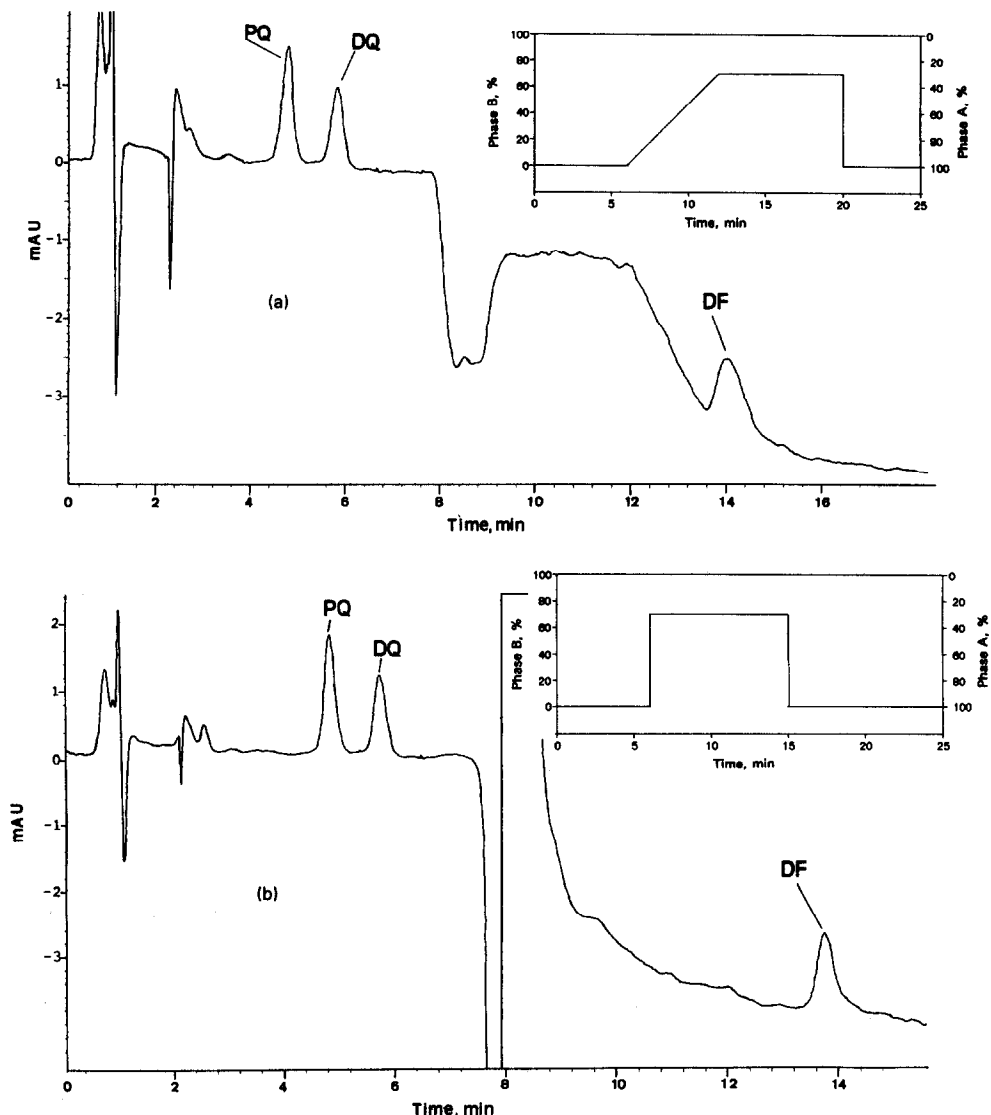
Fig. 1. Chromatogram of a standard solution of PQ (88.1 μg l^{-1}), DQ (101.8 μg l^{-1}) and DF (112.9 μg l^{-1}) in mobile phase A. Mobile phase A: 40 mM SHS, 100 mM H$_3$PO$_4$ and 100 mM TEA (pH 3.0) mixed with ACN (93:7, v/v). Mobile phase B: 40 mM SHS, 100 mM H$_3$PO$_4$ and 100 mM TEA (pH 3.0) mixed with ACN (60:40, v/v).

of ionic species injected into the column thus increased and, therefore, a higher detector response was achieved.

Field-amplified sample injection improved the sensitivity, but variable matrix effects due to different electrolyte concentrations influenced the accuracy and precision. The effect of the electrolyte concentration in the sample solution, *i.e.*, conductivity, was studied. Fig. 3 shows a dramatic decrease in the analytical signal when the NaCl concentration was increased from 10 to 100 mM. As the conductivity of the sample solution increased, the field-amplified sample injection decreased.
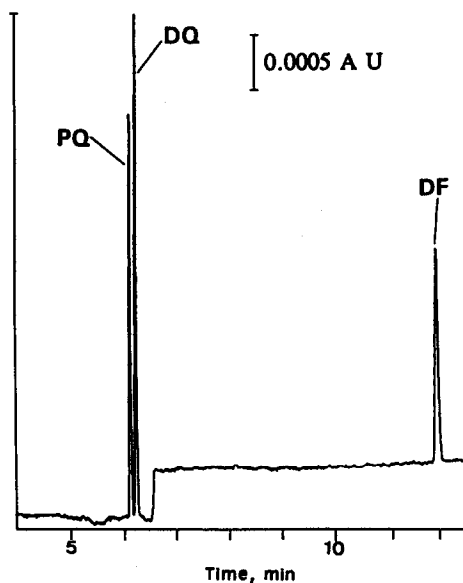
Fig. 2. Electropherogram of a standard aqueous solution of PQ (410 μg l^{-1}), DQ (480 μg l^{-1}) and DF (430 μg l^{-1}). Electrokinetic injection, 10 s.

Fig. 3. Influence of sample conductivity on peak area. Electrokinetic injection, 10 s. Standard solutions: PQ (527 μg l^{-1}), DQ (648 μg l^{-1}) and DF (1132 μg l^{-1}). Figures above the abscissa are the sodium chloride concentrations (mM) in the standard solution injected. $\square$ = PQ; $\blacksquare$ = DQ; $*$ = DF.

Further, it was observed that, using electrokinetic injection, the peak area was influenced by the volume of the sample solution in the injection vial. In Fig. 4 the peak area is plotted against the sample solution volume. When the sample solution volume was less than 200 μl the peak area was significantly reduced. This could be due to the variation of the liquid height above the capillary end, which would produce a change in the plug length of the sample solution inside the capillary. Consequently, poor precision may be obtained unless a measured volume is used.
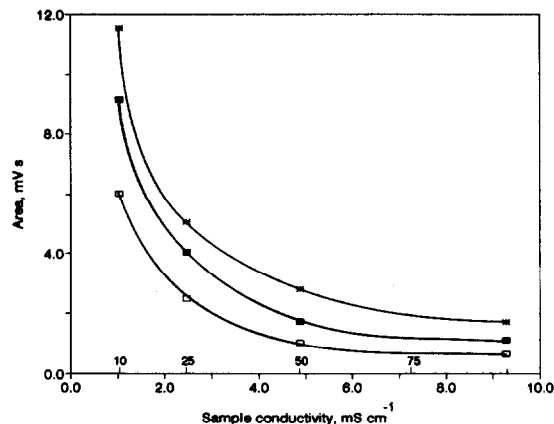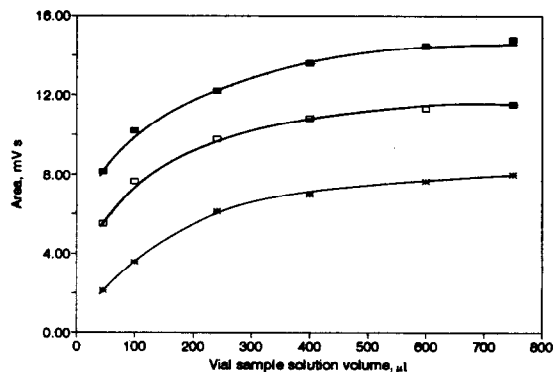

Fig. 4. Effect of vial sample solution volume on peak area. Standard aqueous solution: PQ (78.0 μg l^{-1}), DQ (91.8 μg l^{-1}) and DF (102.2 μg l^{-1}). Electrokinetic injection, 10 s. $\square$ = PQ; $\blacksquare$ = DQ; $*$ = DF.

Table 1
Influence of injection mode on response factors

Solvent	Normalized response factor					
	Electrokinetic mode			Hydrodynamic mode		
	PQ	DQ	DF	PQ	DQ	DF
Water	29.7	34.6	30.0	3.6	4.8	14.2
Running buffer	1.2	1.0	4.0	5.2	5.7	19.5

Table 2
Quality parameters

Parameter	Paraquat		Diquat		Difenzoquat	
	CE[a]	HPLC	CE[a]	HPLC	CE[a]	HPLC
LOD[b] in water (μg l^{-1})	2.9	3.6	2.3	3.2	3.9	5.5
LOD[b] in crop water (μg l^{-1})	21	3.6	18	3.2	31	5.5
Run-to-run reproducibility, R.S.D. (%) ($n = 8$)	5.7	4.0	5.8	2.0	5.2	0.8
Day-to-day reproducibility, R.S.D. (%) ($n = 4$)	6.7	5.7	5.9	5.1	6.5	5.6
Calibration level (μg l^{-1})	10–100	10–1000	10–100	10–1000	10–100	10–1000

[a] Injection mode: electrokinetic (10 s).
[b] 3σ signal-to-noise ratio.

3.3. Quality parameters

Quality parameters using HPLC and CE with electrokinetic injection are given in Table 2. The detection limits, expressed as μg l^{-1} of quaternary ammonium ion, are based on a signal-to-noise ratio of 3:1 and were similar for HPLC and CE when the standards were dissolved in water. For real samples an increase in the detection limits in CE with electrokinetic injection was observed, which could be related to the conductivity of the samples, as mentioned above.

Eight replicate determinations of 80 μg l^{-1} standard solutions of each compound were carried out under the optimum conditions to determine the run-to-run reproducibilities of the two methods. Relative standard deviations (R.S.D.) based on peak area in the range 5.2–5.8% for CE and 0.8–4% for HPLC were obtained. These values were similar but slightly higher for CE. It must be pointed out that in CE it was necessary to control the volume of the sample to maintain the variation at this level; volumes less than 200 μl increased the R.S.D. to 15%.

In order to test the day-to-day reproducibilities of the two methods, four replicate analyses of a standard solution of 80 μg l^{-1} were carried out on four different days. The R.S.D.s in the concentration are given in Table 2 and show that the two methods were similar.

Calibrations for paraquat, diquat and difenzoquat at concentrations between 10 and 1000 μg l^{-1} were carried out. Peak area was used as the response and the correlation coefficients in the intervals of linearity were better than 0.998 for the three compounds. The intervals of linearity for each compound with the two methods are given in Table 2, where it can be observed that they are higher in HPLC than in CE.

3.4. Application

To show the applicability of the methods to the routine analysis of real samples, different irrigation waters were used as samples. The results obtained for three water samples that contained paraquat and two samples spiked with the three compounds are given in Table 3. The results obtained with the two methods were similar, although the R.S.D.s were higher for CE. Waters with paraquat levels lower than the detection limits for CE (samples A, B and E) were concentrated before analysis, but the results were similar to those obtained using HPLC, showing that the concentration step did not interfere in the quantification.

3.5. Features of the techniques

Separation of the three compounds can be achieved with both techniques and the resolution

Table 3
Determination of herbicides in crop waters

Sample	Concentration (μg l^{-1})					
	Paraquat		Diquat		Difenzoquat	
	CE	HPLC[a]	CE[a]	HPLC[a]	CE[a]	HPLC[a]
A	17.2 ± 1.8[b]	16.2 ± 0.6	–	–	–	–
B	14.5 ± 1.2[b]	13.0 ± 1.7	–	–	–	–
C[c]	70.0 ± 4.3[a]	76.4 ± 1.6	82.0 ± 4.0	83.0 ± 1.2	70.0 ± 3.9	75.8 ± 0.8
D[c]	67.0 ± 4.0[a]	70.7 ± 2.9	78.0 ± 4.3	82.0 ± 2.0	68.0 ± 5.0	73.7 ± 1.7
E	21.9 ± 1.6[b]	20.7 ± 1.3	–	–	–	–

Results are means ± standard deviations ($n = 3$).
[a] Standard addition calibration.
[b] Determination after sample concentration (35-fold). External calibration with 50 mM NaCl aqueous standards.
[c] Spiked sample.

between paraquat and diquat was similar (CE: 1,7; HPLC: 2.0). One of the main drawbacks of the CE method was its sensitivity. Although similar detection limits were obtained with the two methods under the optimum conditions, they were greatly affected by the nature of the sample matrix for capillary electrophoresis, because of the saline content of the water, which had an important effect on the amount of solute injected.

The analysis time was similar for both methods (ca. 20 min), but CE capillaries did not require preconditioning; flushing with buffer running solution for 2 h was sufficient. In contrast, in ion-pair HPLC, stabilization of the system for 12 h was needed to obtain good reproducibility.

One of the key points in the comparison is the eluent requirements. In the proposed CE method, the eluent was an aqueous buffer solution, whereas in the ion-pair HPLC method a mobile phase containing acetonitrile, a buffer solution and an ion-pairing agent must be used. Moreover, the eluent consumption in CE was lower than that in HPLC, and also organic solvents were not needed, so important decreases in costs and in environmental pollution was achieved.

Simplicity and ease of operation are two key features of capillary electrophoresis usually stressed by different workers, and here they were especially important owing to the difficulty in obtaining the separation shown in Fig. 1 in routine analyses of real samples.

4. Conclusions

Both capillary electrophoresis and ion-pair reversed-phase liquid chromatography may be used successfully for the determination of paraquat, diquat and difenzoquat. Capillary electrophoresis was simpler and cheaper, but ion-pair reversed-phase chromatography offered a higher sensitivity for real samples and it was independent of matrix effects. For waters with very low herbicide levels, HPLC may be used without previous concentration, but preconcentration (30-50-fold) must be carried out using CE. When the concentration of the herbicides was higher than 18 μg l^{-1}, direct determination with CE gave good results, provided that the conductivity was low enough.

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6. References

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