Journal of Chromatography, 629 (1993) 379–384
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 666

Analysis of anthraquinone sulphonates

Comparison of capillary electrophoresis with high-performance liquid chromatography

Stephen J. Williams and David M. Goodall

Chemistry Department, University of York, York YO1 5DD (UK)

Kenneth P. Evans

ICI Specialties, P.O. Box 42, Blackley, Manchester M9 3DA (UK)

(First received June 17th, 1992; revised manuscript received September 29th, 1992)

ABSTRACT

Capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC) are compared for the analytical separation of anthraquinone-1-sulphonate and its related impurities anthraquinone-2-sulphonate, anthraquinone-1,8-disulphonate and anthraquinone-1,5-disulphonate. Optimum conditions for CE use a borate solution (50 m*M* NaH_2BO_3 and 8 m*M* H_3BO_3) as background electrolyte at pH 10.0, and for HPLC acetonitrile–water (60:40) containing 0.8% (w/v) cetyltrimethylammonium bromide as eluent. Analysis times are comparable by both techniques. Quantitative aspects including CE and HPLC efficiency, linearity, precision and limits of detection (LOD) are compared. For anthraquinone-1-sulphonate the CE concentration LOD is 0.7 $\mu g\ ml^{-1}$ and the HPLC LOD is 6 $ng\ ml^{-1}$; mass LODs are 3 and 60 pg, respectively. Assay precision is 2% R.S.D. in peak area by CE using instruments from several manufacturers. This is improved to better than 0.5% R.S.D. in relative peak areas when a co-injected analyte is used as an internal standard, showing that injection is the factor limiting precision in CE. Integration errors give a significant contribution to the observed error in relative peak areas.

INTRODUCTION

Of increasing interest in capillary electrophoresis (CE) [1,2] are quantitative aspects of the technique [3,4] and its performance relative to established separation methods. For small molecules CE can provide robust assays, but for large molecules which are particularly subject to sample matrix effects and capillary wall interactions the full potential of CE has yet to be established. Reproducibility of sample injection is a key determinant of accuracy and precision in absolute quantitation whilst detector sensitivity constrains dynamic range, accuracy and limit of detection [5,6]. Other factors which must be carefully controlled are background electrolyte composition [5,7] and pH [8], capillary surface condition [7,9], and capillary temperature [10].

In this paper we report a systematic study of the analysis of anthraquinone-1-sulphonate and related impurities, and compare CE results with those achievable by HPLC.

EXPERIMENTAL

Instrumentation

Four commercial CE instruments were used in this study: (I) Applied Biosystems (ABI) 270A; (II)

Correspondence to: D. M. Goodall, Chemistry Department, University of York, York YO1 5DD, UK.

0021-9673/93/$06.00 © 1993 Elsevier Science Publishers B.V. All rights reserved

Beckman P/ACE 2000; (III) Spectra-Physics Spectrophoresis; (IV) Waters Quanta 4000. II and IV were fitted with 75 μm internal diameter (I.D.) fused-silica capillaries, whilst I and III had 50 μm I.D. capillaries. In all cases samples were introduced by hydrodynamic injection at the anodic end of the capillary and detected by UV absorbance in the range 254–260 nm. Electrophoresis was carried out at applied voltages of 20 kV for I, II, IV and 15 kV for III. Corresponding field strengths were: I, 280; II, 350; III, 360; IV, 330 V cm^{-1}. Temperatures set were 30°C for I and II and 25°C for III, whilst IV operated at ambient temperature.

HPLC linearity data were obtained using a system comprising a pump (Altex 110A), autosampler (Perkin-Elmer 420), UV detector (Perkin-Elmer 75T) and integrator (Hewlett-Packard 3354). All other HPLC analyses were carried out using a chromatograph (Hewlett-Packard HP 1090L) fitted with a diode array detector (HP 1040). The detection wavelength was 254 nm and bandwidth 4 nm. A 10-μl injection volume and a 25 cm × 4.5 mm ODS Hypersil column at 40°C were used for all HPLC experiments.

Materials

The anthraquinone sulphonates used were obtained from various sites within the ICI Fine Chemicals Manufacturing Organisation. Compounds and their purities were: sodium anthraquinone-1-sulphonate (96%), sodium anthraquinone-2-sulphonate (97%), sodium anthraquinone-1,5-disulphonate (82%), and potassium anthraquinone-1,8-disulphonate (75%). The CE background electrolyte found to give optimum separation was a borate buffer at pH 10.0 (50 m*M* Na_2HBO_3 and 8 m*M* H_3BO_3) prepared from sodium tetraborate and sodium hydroxide solutions. The HPLC eluent found to give optimum separation was acetonitrile–water (60:40) containing 0.8% (w/v) cetyltrimethylammonium bromide (CTAB). The acetonitrile was of HPLC grade (Rathburn) with CTAB obtained from ICI Pharmaceuticals.

RESULTS AND DISCUSSION

Separations

CE separations of the four anthraquinone sulphonates (Fig. 1) were carried out over a range of pH values. Fig. 2 shows the optimised separation using borate as background electrolyte at pH 10. The low pK_a of the sulphonic acid group ensured that the analytes were negatively charged at all pH values investigated and migrated against the electroosmotic flow. The electroosmotic flow has greater magnitude than the electrophoretic mobilities of the analytes, and their migration times to the detector are in reverse order to the magnitude of their electrophoretic mobilities. The electrophoretic mobility order is 4 > 3 > 2 > 1, with the dianions having higher values than the monoanions as expected, and baseline resolution was obtained for all peaks. Reversal of the polarity coupled with operation at low pH (20 m*M* sodium citrate, pH 2.5) resulted in all sulphonates eluting in under 6 min. However, resolution was found to be much worse than in the high pH case and unsuitable for quantitative studies.

Fig. 3 shows the optimised HPLC separation. The order of elution can be explained in part by considering the effect of ion pairing with CTAB. The greater the interaction the molecules have with the cationic surfactant the more hydrophobic they become, and the greater their retention on the C_{18} stationary phase. The disulphonates form stronger ion pairs than the monosulphonates, and the greater local charge density on the 1,8-dianion than on the 1,5-dianion explains why anthraquinone-1,8-disulphonate forms the strongest ion pair and elutes last.

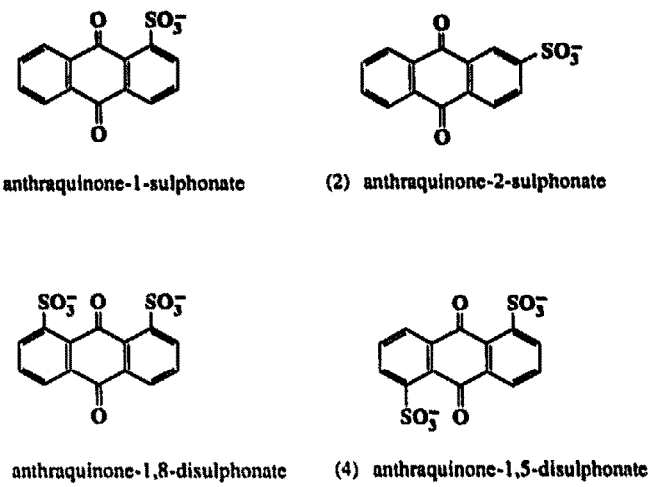

Fig. 1. Structures of (1) anthraquinone-1-sulphonate, (2) anthraquinone-2-sulphonate, (3) anthraquinone-1,8-disulphonate, (4) anthraquinone-1,5-disulphonate.

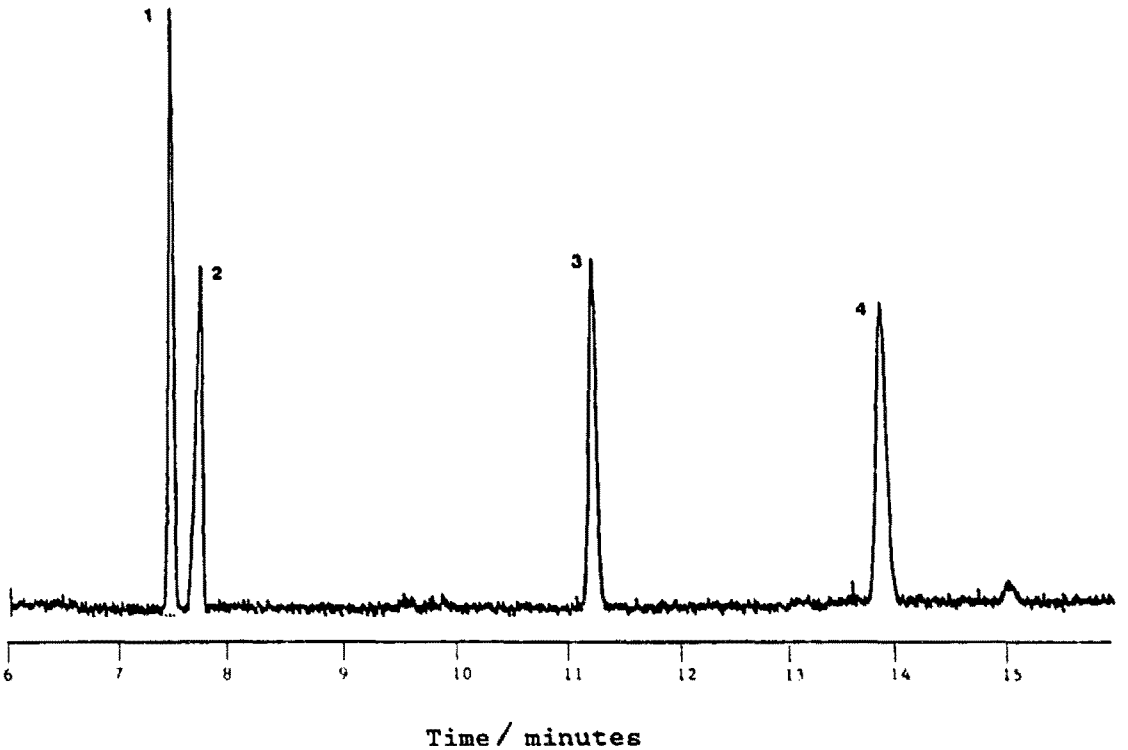

Fig. 2. Electropherogram of anthraquinone sulphonates. Conditions: buffer, 50 m*M* NaH_2BO_3 and 8 m*M* H_3BO_3 pH 10.0; capillary, 72 cm (50 cm to detector) × 50 μm I.D.; applied voltage, 20 kV; temperature, 30°C; detection, UV absorbance at 254 nm; injection, 1 s vacuum. Sample concentration 0.1 mg ml^{-1}.

Efficiency

In comparing the CE and HPLC separations in Figs. 2 and 3, it should be noted that the analysis times are comparable (14–17 min), although HPLC has better resolution for peaks 1 and 2 and could be made at least twice as fast whilst still retaining good resolution between these two peaks. CE has better peak shape and efficiency. With efficiency defined in terms of number of theoretical plates, *N*,

$$N = 5.54\ l^2/w^2 \quad (1)$$

where *l* is the length to the detector and *w* the peak width at half height, CE efficiencies are in the range $1.2 \cdot 10^5$–$6.7 \cdot 10^4$ theoretical plates compared to HPLC efficiencies of $1.8 \cdot 10^4$–$2.6 \cdot 10^3$ theoretical plates.

Linearity

For anthraquinone-1-sulphonate, plots of peak area *versus* concentration over the range 0–350 μg ml^{-1} were made to determine linearity for both HPLC and CE. Correlation coefficients were found to be 0.99995 and 0.99999, respectively. Excellent correlation coefficients were obtained on all CE systems when using peak area. In all cases plots of peak height *versus* concentration showed curvature indicative of peak broadening above an anthraquinone-1-sulphonate concentration of 50 μg ml^{-1}. This value is in the lower part of the linear range for the analyte, and emphasises, as has been noted elsewhere [6], the importance of using peak area rather than peak height in quantitative studies using CE.

Limit of detection

Table I compares limits of detection (LOD) for CE and HPLC, with concentration LOD values taken with the ratio of signal to peak-to-peak noise level equal to 2:1. The primary data for the CE analysis are given in Fig. 4; the peak-to-peak noise is $1 \cdot 10^{-4}$ AU at 254 nm, using a risetime of 1 s, and the LOD for anthraquinone-1-sulphonate is 0.7 μg

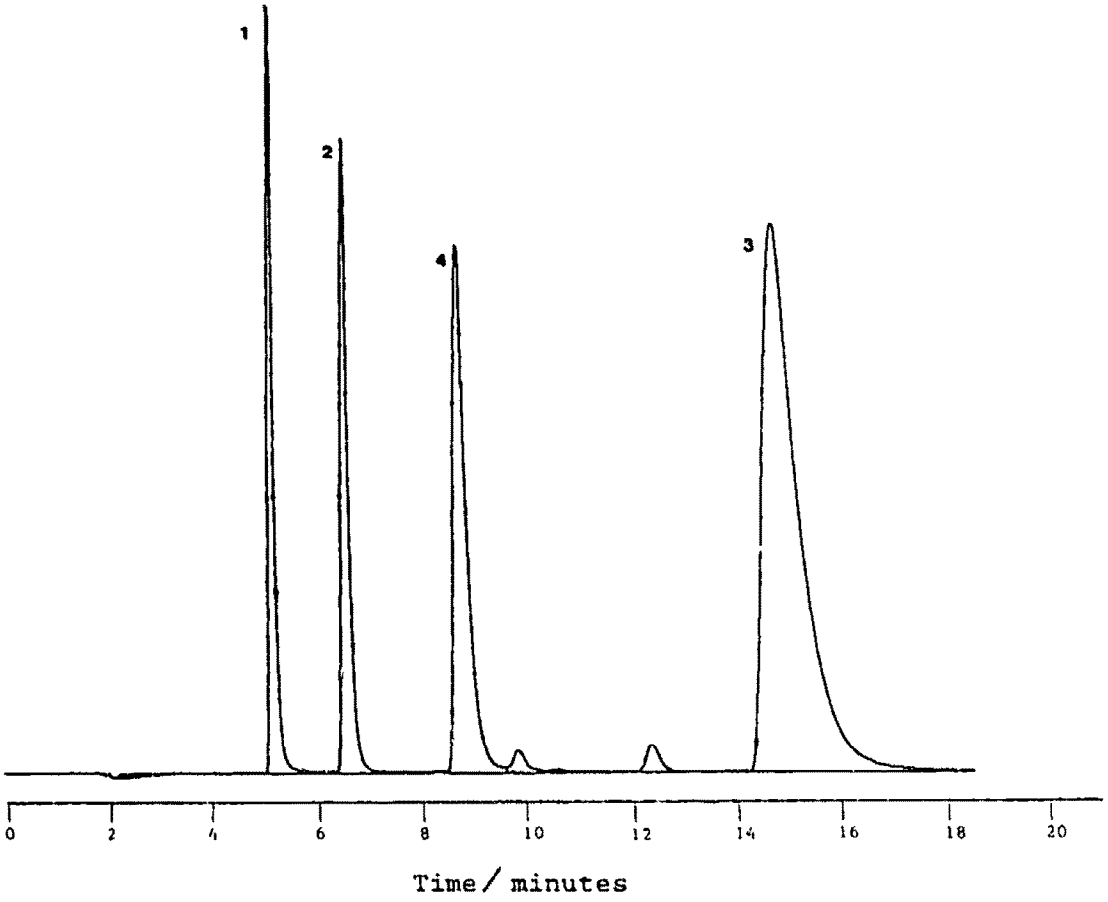

Fig. 3. Chromatogram of anthraquinone sulphonates. Conditions: eluent, acetonitrile–water (60:40) containing 0.8% (w/v) CTAB; column, 25 cm × 4.5 mm ODS Hypersil; flow-rate, 1 ml min^{-1}; temperature, 40 C; detection, UV absorbance at 254 nm; injection, 10 μl. Sample concentration 0.1 mg ml^{-1}.

ml^{-1} ($2 \cdot 10^{-6}$ *M*). To derive accurate mass LOD values in CE it is vital to know accurately the injection volume. For the ABI instrument used this has been determined by microscopic measurement of water displacement by air with correction for surface tension, and measured values were found to agree well with those calculated using the Poiseuille equation [6].

A criticism frequently levelled at CE is that whilst its mass detection limits are good due to the small injection volume, compared with HPLC its concentration limits of detection are poor. This is born out by the data in Table I, where it can be seen that the mass LOD is 20 times better for CE than for HPLC, but that HPLC can detect a 100 times less concentrated sample. Concentration LOD differences using absorbance detection are essentially a function of the difference in optical path lengths between the HPLC detector flow cell (1 cm) and the CE capillary ($5 \cdot 10^{-3}$ cm).

TABLE I

CONCENTRATION AND MASS DETECTION LIMITS FOR ANTHRAQUINONE-1-SULPHONATE

Method	Injection volume (μl)	Concentration detection limit (μg ml^{-1})	Mass detection limit (pg)
CE	0.004	0.7	2.8
HPLC	10	0.006	56

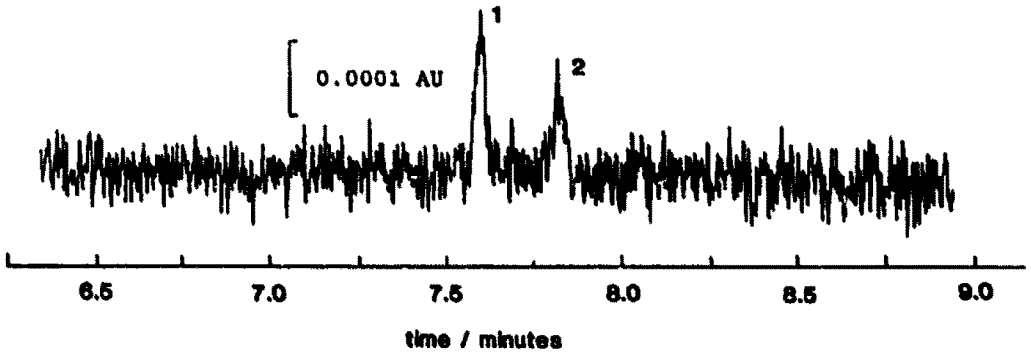

Fig. 4. Electropherogram of (1) anthraquinone-1-sulphonate and (2) anthraquinone-2-sulphonate used to determine LOD. Conditions as in Fig. 2, except sample concentration 1.6 $\mu g\ ml^{-1}$.

Precision

For studies of precision in the HPLC method 15 injections of sample were made at a concentration of 0.1 mg ml^{-1}. The relative standard deviation (R.S.D.) in peak area was found to be 0.5%.

Representative results of studies of reproducibility of migration time and peak areas in CE are given in Table II. Since the area of a peak is proportional to its migration time [11], improved peak area R.S.D.s are expected to result after normalising peak areas for migration times. R.S.D.s obtained from data based on 33 replicate injections on one instrument do indeed improve on normalisation (bracketted values), consistent with errors in peak area and migration time being correlated. In contrast to this, with another instrument the area R.S.D.s are not improved by normalisation and the errors appear to be uncorrelated. Correlated errors may be evident in a series where there is drift in retention time (*e.g.*, using a new capillary [9]).

After normalisation, peak area R.S.D.s are 2% for all anthraquinone sulphonates on both instruments. The last column in Table II shows the effect of ratioing the peak area of the anthraquinone-1-sulphonate to that of a second component in the mixture of analytes injected. R.S.D.s in relative peak areas are up to a factor 7 better than R.S.D.s in absolute peak areas, and the removal of all correlated errors as shown in Table II yields very good R.S.D. values, less than 0.5%. This proves that injection is the dominant source of error, limiting peak area reproducibility to 2% in the present generation of automated CE instruments. If it is desired to quantitate analytes to a higher degree of

TABLE II

REPRODUCIBILITY OF MIGRATION TIMES AND PEAK AREAS

Instrument	Analyte	R.S.D. (%)		
		Migration time	Peak area[c]	Peak area (1) / Peak area (4)
II[a]	(1) Anthraquinone-1-sulphonate	0.5	1.9(1.9)	0.4
	(4) Anthraquinone-1,5-disulphonate	0.7	2.1 (2.6)	
III[b]	(1) Anthraquinone-1-sulphonate	0.9	2.2 (2.0)	0.9 (0.3)
	(4) Anthraquinone-1,5-disulphonate	1.7	3.1 (2.2)	

[a] Data based on 17 replicate injections.
[b] Data based on 33 replicate injections.
[c] Normalized peak areas in brackets.

precision, either multiple injections (to decrease the standard error on the mean) or the use of an internal standard is required.

One factor often overlooked in quantitative studies in CE is the effect of integration on area determination. Grushka and Zamir [12] have shown that for a Gaussian peak the relative error in the area, $\delta A/A$, is given by

$$\frac{\delta A}{A} = \frac{4}{\sqrt{2\pi}} \left(\frac{S}{N}\right)^{-1} \frac{w^{3/2}}{n} \quad (2)$$

where S/N is the ratio of signal to root-mean-square noise, w is the width of the peak at half height, and n is the number of data samplings across this width [12]. Inserting values obtained using an integration rise time of 1 s (3.3 data points per second) and analyte concentration 0.1 mg ml^{-1} gives a calculated contribution to the relative peak area R.S.D. using the 1,5-disulphonate as internal standard for anthraquinone-1-sulphonate of *ca.* 0.2%, *i.e.*, a significant contribution to the observed values of 0.3–0.4% given on the last column of Table II.

CONCLUSIONS

The comparison of CE with HPLC for the analysis of anthraquinone-1-sulphonate and three related impurities shows that both techniques give baseline-resolved separations within 17 min. CE gives better peak shape and efficiency, but HPLC has the better resolution for anthraquinone-1-sulphonate and its -2- isomer. Whereas CE is superior in its mass limit of detection, HPLC has an advantage of two orders of magnitude in concentration limit of detection. Excellent correlation coefficients are obtained for peak area as a function of concentration for both techniques Assay precision is 0.5% R.S.D. in peak area by HPLC and 2% by CE, though the latter is improved to better than 0.5% R.S.D. using an internal standard. Closely comparable figures were obtained on CE instruments from four different manufacturers, showing that injection is the factor limiting precision in every case. The use of an internal standard is therefore recommended for high-precision CE analyses. An analysis of errors in integration in CE shows that these are significant at the 0.2% R.S.D. level, and it will be advisable to work at the highest practicable analyte concentration and at a high digitisation rate when seeking the highest precision in CE.

ACKNOWLEDGEMENTS

We wish to acknowledge support from the SERC for an SERC CASE studentship to S.J.W., and assistance from Applied Biosystems, Beckman, Spectra Physics and Waters for the use of CE instrumentation.

REFERENCES

1 R. A. Wallingford and A. G. Ewing, *Adv. Chromatogr.*, 29 (1989) 1.
2 W. G. Kuhr, *Anal. Chem.*, 62 (1990) 403R.
3 D. M. Goodall, S. J. Williams and D. K Lloyd, *Trends Anal. Chem.*, 10 (1991) 272.
4 E. B. Dose and G. A. Guiochon, *Anal. Chem.*, 63 (1991) 1154.
5 D. J. Rose and J. W. Jorgenson, *Anal. Chem.*, 60 (1988) 642.
6 S. E. Moring, J. C. Colburn, P. D. Grossman and H. H. Lauer, *LC · GC*, 8 (1) (1990) 34.
7 M. Zhu, R. Rodriguez, D. Hansen and T. Wehr, *J. Chromatogr.*, 516 (1990) 123.
8 K. Salomon, D. Burgi and J. C. Helmer, *J. Chromatogr.*, 549 (1991) 375.
9 D. K. Lloyd, *Anal. Proc.*, 29 (1992) 169.
10 J. H. Knox and I. H. Grant, *Chromatographia*, 61 (1987) 24.
11 X. Huang, W. F. Coleman and R. N. Zare, *J. Chromatogr.*, 480 (1989) 95.
12 E. Grushka and I. Zamir *Chem. Anal. (N.Y.)*, 98 (1989) 529.