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Comparison of capillary electrophoresis systems for the reliable identification of some carboxylic acids using the marker technique

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Abstract

Many commercial capillary electrophoretic (CE) systems differing in degree of automation, type of injection and capillary cooling are available. The aim of this study was to investigate if the marker technique can be used for reliable peak identification of compounds separated with different CE instruments. In the marker technique, which was developed earlier in our laboratory, unknown compounds are identified through determination of their electrophoretic mobilities relative to the known mobilities of the marker compounds. Carboxylic acids were used as markers and model components and a phenolic compound was added to the sample mixture as a pH-sensitive probe. The repeatabilities and reproducibilities of both the migration times and the mobilities of the analytes were measured with five different CE instruments. With the electrophoretic mobilities, excellent repeatability and reproducibility were obtained. © 1997 Elsevier Science B.V.

Keywords: Electroosmotic flow; Electrophoretic mobility markers; Marker technique; Carboxylic acids

1. Introduction

Capillary electrophoresis (CE) has rapidly gained popularity among researchers in various fields, and many commercial CE systems are already available. Manufacturers tend to improve their systems not only by designing easy-to-use software programs with improved signal handling, but also by equipping their instruments with more reliable injection and capillary cooling systems. Equipment is becoming more user-friendly and easier to use, and it is now possible to connect CE instruments to many other analytical instruments.

Electroosmotic flow commences whenever an electric field is applied to a capillary filled with a conductive electrolyte solution. In uncoated capillaries, the electroosmotic flow (EOF) increases with pH, and the greatest change in the mobility of analytes usually occurs between pH values of about 5 and 7. Processes such as ion adsorption, ion exchange or gel layer formation may change the EOF by altering the zeta potential on the capillary wall [1]. The difficulty in obtaining repeatable EOFs between different capillaries and between different runs in the same capillary is a matter of concern to many CE users, because variation in EOF is the main cause of unrepeatable and irreproducible migration times. Both the magnitude and strength of the EOF affect the migration times, and also the resolution of compounds. Dealing with the EOF velocity (v_{eo}) is thus essential for the characterization of analytes in a CE separation.

Several approaches to controlling the EOF have been proposed, e.g. altering the chemical or physical

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properties of the electrolyte solution, coating the capillaries, and applying an external electric field [2]. Alternatively, rather than suppressing it, attempts have been made to take the v_{eo} into account by determining its time dependency [3–5]. One of these techniques, recently developed in our laboratory, relies on marker compounds of known electrophoretic mobility, and on the effective electric field strength [4,6,7]. Mobilities of the marker compounds remain constant despite small changes that may occur in the electrolyte solution. A good marker compound will have a pK_a value that is far from the pH of the buffer and will not undergo degradation or conformational change throughout one run. The effective electric field strength used in the calculations of the mobilities is the net force affecting an ion during an electrophoretic run. With markers included in the sample mixture, mobilities of unknown compounds can be calculated with high precision: relative standard deviations (R.S.D. values) of below 0.1% have been obtained. The marker technique has also proved to be highly precise for peak identification. In addition, the electrophoretic mobilities of analytes calculated with marker compounds can be utilized for studies of quantitative structure–electrophoretic mobility relationships (QSEMR).

The marker technique, which has been applied to the identification of both small ions [8] and macromolecules [9], eliminates the negative effects of Joule heating, electroosmosis, variations in capillary length and in the separation power [10]. The temperature effects in capillaries are of particular importance due to their influence on several properties in the electrophoretic system. Heating of the capillary, due to the passage of current, i.e. Joule heating, decreases the viscosity of the buffer, which leads to an increase in the measured electroosmotic mobility. Cooling of the capillary will minimize the temperature gradients in the capillaries and keep the flow profile plug-like. Also, the charge and the pK_a value of an analyte may change if the temperature varies. If the pK_a value of an analyte is close to the pH of the buffer, Joule heating effects may cause changes in the pH or the pK_a value of the buffer and these, in turn, may affect the charge of the analyte.

All CE instruments differ from each other in one way or another, and there are even slight differences between instruments from the same manufacturer. We wanted to know whether or not the marker technique can be used to measure the reliability of peak identification obtained with different CE instruments. In other words, we wanted to investigate the reproducibility of the marker technique. Five instruments were used in the study, two of them differing only in age. Different types of pressurized injection and different integration modes for data recording were used with the instruments. We also wished to see how well the marker technique can compensate for differences in sample introduction into the capillary. Yet another essential difference between the instruments, besides the injection system, was the cooling of the capillaries. From the discussion above, it is clear that temperature has a marked influence on several properties in an electrophoretic separation. Thus, one additional goal was to determine whether or not repeatable mobilities can be obtained with the marker technique for compounds separated with instruments lacking temperature control units. Even in instruments that do have temperature control units, the temperature varies in unthermostated segments of the capillaries, and this might lead to irreproducibilities in the mobilities of the analytes.

In an earlier publication [11], it was also established that highly repeatable results are obtained with the marker technique if buffer replenishment is applied. Accordingly, in this study, we introduced fresh electrolyte solution for each run. Eleven carboxylic acids (seven analytes and four markers) were chosen as model compounds for the investigation of the peak identification method, irrespective of the CE instrument used. A phenolic compound with a pK_a value close to the buffer pH was added to the test mixture as a pH-sensitive probe. Some data on repeatabilities and reproducibilities of absolute migration times and of the mobilities of all of the compounds, separated with different CE instruments, are presented.

2. Theory

2.1. Effective electric field strength

In the marker technique, compounds of known electrophoretic mobilities are used to determine the

effective electric field strength (E_{eff}) and electroosmotic flow velocity (v_{eo}) of a system. Due to the charge asymmetry effect, the electrophoretic effect and inhomogeneities in the buffer, there will be a net force, E_{eff}, affecting an ion moving in a strong electric field. It has been shown that E_{eff} is approximately 80% of the electric field strength (E) when 80 m*M* 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS) buffer is used [7]. Thus

$$E = U/L_{tot} \quad (1)$$

and

$$E_{eff} \leq E, \quad \lim(I \rightarrow 0)E_{eff} = E \quad (2)$$

where U is the voltage and L_{tot} the total length of the capillary. The buffer composition has a marked effect on the ratio of E_{eff} to E [12].

2.2. The marker techniques

The two, three and four marker (2m, 3m and 4m) techniques were applied and compared in this study. Here, E_{eff} and v_{eo} are calculated from the known electrophoretic mobilities and absolute migration times of the marker compounds, and from the length of the capillary to the detection window. With all marker techniques, it is approximated that E_{eff} stays constant during one run. In the case of 2m, v_{eo} is also approximated to remain constant during a single run,

$$v_{eo} = a \quad (3)$$

The distance an analyte travels in the capillary, as a function of time, is expressed as

$$s = \int v_{tot} \mathrm{d}t \quad (4)$$

where $s = L_{det}$. Accordingly, the electrophoretic mobilities can be derived from these equations and calculated from

$$\mu_{ep(x)} = (L_{det}/t_x - a)/E_{eff} \quad (5)$$

With the 3m technique, v_{eo} is approximated to be linearly accelerating,

$$v_{eo} = bt + a \quad (6)$$

and the electrophoretic mobilities for the analytes are

$$\mu_{ep(x)} = (L_{det}/t_x - bt_x/2 - a)/E_{eff} \quad (7)$$

In 4m techniques, v_{eo} is approximated to be non-linearly accelerating,

$$v_{eo} = ct^2 + bt + a \quad (8)$$

The corresponding equation for the electrophoretic mobilities of the analytes can then be calculated from

$$\mu_{ep(x)} = (L_{det}/t_x - ct_x^2/3 - bt_x/2 - a)/E_{eff} \quad (9)$$

Due to the complexity of the matrices involved in the prediction of the electrophoretic mobilities, computer-based programs are recommended for processing the data, especially when using 3m or 4m. The marker technique is described in detail elsewhere [4].

3. Experimental

3.1. Chemicals

3-(Cyclohexylamino)-1-propanesulfonic acid (CAPS), diphenylacetic acid, probenecid and ethacrynic acid were obtained from Sigma (St. Louis, MO, USA); benzoic acid was from Fluka (Buchs, Switzerland); triphenylacetic acid was from Aldrich (Steinheim, Germany); HPLC-grade methanol, *o*-cresol and NaOH were from Merck (Darmstadt, Germany); xanthene-9-carboxylic acid and mandelic acid were from EGA Chemie (Steinheim, Germany); phenylacetic acid was from BDH (Poole, UK) and *meso*-2,3-diphenylsuccinic acid was from TCI (Tokyo, Japan). 1,2-Phenylenediacetic acid and *o*-phthalic acid were synthesized in the Laboratory of Organic Chemistry at the University of Helsinki (Helsinki, Finland). Distilled water was further purified with a Water-I system (Gelman Sciences, Ann Arbor, MI, USA). Before electrophoresis, the buffer was filtered through 0.45 μm Acrodisc PTFE membrane filters (Gelman Sciences).

3.2. Apparatus

Separations were performed with five different CE instruments. Uncoated fused-silica capillaries were 50 μm I.D. and 360 μm O.D. (Composite Metal Services, The Chase, Hallow, UK). In all cases, the length of the capillary to the detector was 70 cm,

while the total length varied with the demands of the instrument. A new capillary was used in each instrument. The type of injection and the injection time varied. Before injection, the capillaries were rinsed for 2 min with the buffer solution. The applied voltage was 20 kV; the temperature was 25°C, except in the Waters Quanta 4000 instrument, which lacks a temperature control unit.

1. In the two Hewlett-Packard instruments (HP 3D-CE ChemStation, Rev. A.03.03), the capillary is cooled by air. The total capillary length was 78.5 cm and injection was for 5 s at 50 mbar.
2. The total length of the capillary in the Waters Quanta 4000 instrument was 77 cm. Injection was made by siphoning for 30 s at a height of 10 cm. The integrator was a Hewlett-Packard, HP3392A.
3. In the Beckman (P/ACE 2050) instrument, the capillary is cooled by liquid. The total length of the capillary was 77 cm. Injection was made by applying a pressure of 35 mbar for 5 s. A Hewlett-Packard 33396A integrator was used for data recording.
4. In the Bio-Rad equipment (BioFocus 3000 Operating Software, Version 5.01), the total length of the capillary was 74.6 cm. Injection was performed for 3 s at 70 mbar.

3.3. Samples and buffers

The sample contained 10 μg/ml of each compound (eight analytes and four markers) in a methanol–water mixture (10:90, v/v). The total volume of the sample varied with the instrument, but the concentrations were constant. The buffer was prepared from 40 ml of a 0.2 *M* CAPS solution, 40 ml of 0.1 *M* NaOH and 20 ml of purified water. The pH of the 80 m*M* CAPS buffer was 10.6. Before each run, the buffer was filtered and degassed ultrasonically. The buffer and sample vials were rinsed with HPLC-grade methanol and dried under nitrogen before use. Normally when using the replenishment technique in the HP instruments, the same pair of buffer vials were filled and emptied before each run, which means that there will always be a small amount of the old buffer left in the vials. We chose instead to use nine pairs of cleaned vials, one for each run. Similarly, with the other instruments, a new pair of buffers was used for each injection.

4. Results and discussion

Eleven carboxylic acids and one phenol were chosen as the analytes and markers. Fig. 1 shows the electropherogram for the separation of the compounds on the HP1 instrument. The pK_a values of the carboxylic acids differed greatly from the pH of the buffer, which was adjusted to 10.6. The pK_a value of CAPS is 10.4 and its buffering range is from pH 9.5 to 11.2. All carboxylic acids were totally dissociated and negatively charged and, consequently, small pH fluctuations did not influence their mobilities. The poor repeatability of the EOF is the major cause of the irrepeatable absolute migration times in replicate runs. *o*-Cresol, added to the test mixture as a pH-sensitive probe, has a pK_a value of 10.2, which is very close to the pH of the buffer. At this pH, *o*-cresol is only 60% dissociated, which means that even a small change in the buffer pH could have a dramatic effect on its electrophoretic mobility.

Usually in CE analyses, the capillaries are conditioned for about 20 min with a potassium- or sodium hydroxide solution and also for 20 min with water. The capillary is then often rinsed for about 10 min with the buffer solution. With this kind of pretreatment, it is assumed that the silanol groups on the capillary walls are totally dissociated and that the system is stabilized, meaning that the EOF will be more or less constant. In this study, the capillaries were conditioned for not more than 5 min each with 0.1 *M* KOH and water, and for not more than 2 min with the buffer solution, because variations in v_{eo} were of interest to us. For the same reason, i.e. we wanted to know if the marker technique can correct for non-uniform v_{eo}, the first three runs were the most interesting ones. Nine consecutive runs were used for the calculations of the mobilities and migration times in replicate analyses.

The average migration times of the analytes in nine consecutive runs and the R.S.D. values for each analyte are presented in Table 1. The R.S.D. values varied between 0.5 and 3.6%. Such large variations in the migration times from run to run are typical and are due to changes in v_{eo}. In all instruments, there was an increase in the R.S.D. value of the migration times from the first to the last migrating component, due to the increasing relative effect of variation in v_{eo} on the total velocity of the analyte.

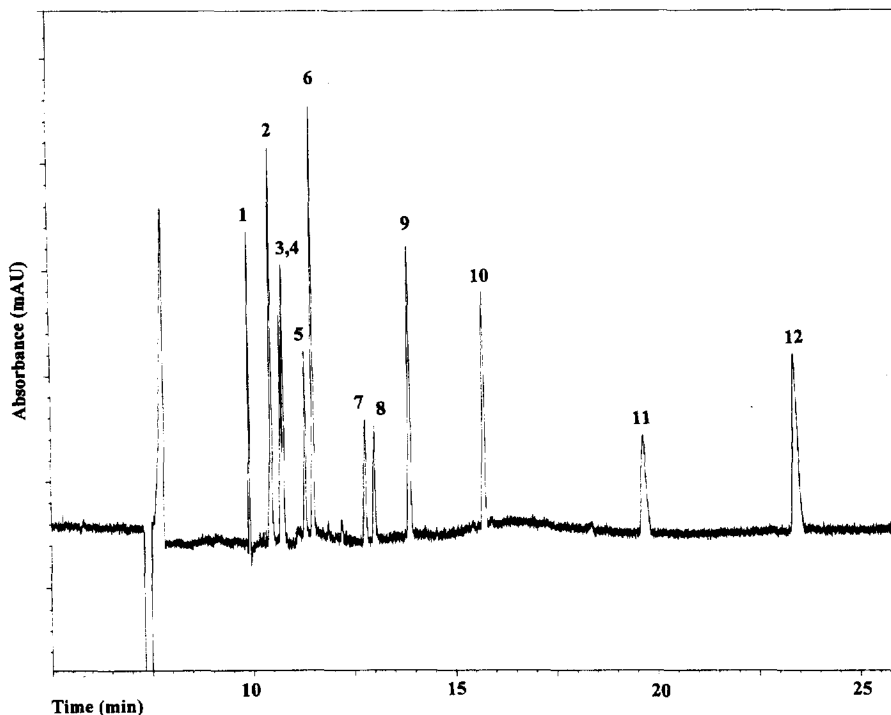

Fig. 1. Separation of eleven carboxylic acids and one phenolic compound (concentration of 10 ppm each) with HP1 equipment. Separation conditions: 70/78.5 cm capillary, 20 kV, 15 μA, 25°C, 220 nm, injection for 5 s at 50 mbar. Numbering of compounds: (1) *o*-cresol, (2) *triphenylacetic acid*, (3) probenecid, (4) ethacrynic acid, (5) *diphenylacetic acid*, (6) xanthene-9-carboxylic acid, (7) mandelic acid, (8) phenylacetic acid, (9) *benzoic acid*, (10) *meso*-2,3-diphenylsuccinic acid, (11) 1,2-phenylenediacetic acid and (12) *o-phthalic acid*. The marker compounds are in italics.

Earlier studies have shown that the migration times can be corrected by the use of mobilities calculated with marker components. Table 2 gives the data on the mobilities and the R.S.D. values for all analytes calculated by the 4m technique, and the results confirm earlier ones. It is worth mentioning that low R.S.D. values for the mobilities of the compounds were obtained even with the Waters Quanta instrument, which does not have a cooling system.

Very often the R.S.D. values of the mobilities are improved when several (i.e. four) markers are used for the calculations, but sometimes, 4m may over-approximate a system, leading to larger deviations. triphenylacetic acid and benzoic acid were the marker compounds in the 2m technique, and diphenylacetic acid and *o*-phthalic acid were the additional compounds in the 4m technique. The 2m technique gave good R.S.D. values for the mobilities (*o*-cresol, 0.43–2.29%; all other compounds, 0.02–0.29%), although the values were not as low as with the 4m technique. In 3m, four different combinations of the four markers used in 4m were tested, i.e. (a) triphenylacetic acid, diphenylacetic acid and benzoic acid; (b) triphenylacetic acid, diphenylacetic acid and *o*-phthalic acid; (c) diphenylacetic acid, benzoic acid and *o*-phthalic acid; (d) triphenylacetic acid, diphenylacetic acid and *o*-phthalic acid. The R.S.D. values for the mobilities calculated by 3m were almost as good as with 4m, which means that, under carefully optimized conditions, three markers in the sample mixture is enough. The greatest difference in the four above cases was for (a): as long as *o*-phthalic acid was included as one of the markers for calculations of mobilities, the R.S.D. values of the two last-migrating analytes were improved and as

Table 1
Average of the migration time (in min) and the corresponding R.S.D. values ($n=9$) of the analytes, measured with different instruments

Compound	HP1		HP2		Waters		BioRad		Beckman	
	Time[a]	R.S.D. (%)	Time	R.S.D. (%)	Time	R.S.D. (%)	Time	R.S.D. (%)	Time	R.S.D. (%)
o-Cresol	10.03	0.748	10.58	1.346	9.96	0.628	9.11	0.756	10.31	0.494
Triphenylacetic acid[b]	10.57	0.920	11.06	1.373	10.50	0.803	9.73	1.229	10.80	0.699
Probenecid	10.77	0.930	11.32	1.401	10.73	0.804	9.94	1.359	11.04	0.715
Ethacrynic acid	10.83	0.938	11.39	1.408	10.79	0.827	9.99	1.238	11.11	0.723
Diphenylacetic acid[b]	11.37	0.962	11.96	1.474	11.31	0.845	10.47	1.304	11.66	0.760
Xanthene-9-carboxylic acid	11.56	0.967	12.17	1.497	11.49	0.844	10.63	1.309	11.86	0.770
Mandelic acid	12.83	1.027	13.53	1.661	12.72	0.903	11.75	1.479	13.17	0.877
Phenylacetic acid	13.05	1.048	13.78	1.695	12.94	0.919	11.95	1.490	13.41	0.898
Benzoic acid[b]	13.92	1.048	14.71	1.823	13.76	0.952	12.68	1.622	14.31	0.951
meso-2,3-Diphenylsuccinic acid	15.68	1.112	16.61	2.098	15.43	0.987	14.20	1.863	16.14	1.089
1,2-Phenylenediacetic acid	19.57	1.099	20.89	2.789	19.00	1.016	17.44	2.429	20.18	1.029
o-Phthalic acid[b]	23.11	1.000	24.86	3.563	22.18	0.874	20.53	2.851	23.96	1.55

[a]Migration times in minutes ($n=9$).
[b]Marker compounds.

good as with 4m. Adding or removing any other marker did not result in notable changes in the R.S.D. values.

Except for the 3m case without *o*-phthalic acid and for *o*-cresol, the repeatabilities of the markers were much improved over the repeatabilities of the migration times. The R.S.D. values with markers were between 0.01 and 0.41%. In the 3m case without *o*-phthalic acid, the values varied between 0.01 and 1.01%. With all marker techniques, R.S.D.

Table 2
Average mobilities ($n=9$) of the analytes, obtained with the 4m technique and five different instruments, and the corresponding R.S.D. values

Compound	HP1		HP2		Waters		BioRad		Beckman	
	Mobility[a]	R.S.D. (%)	Mobility	R.S.D. (%)	Mobility	R.S.D. (%)	Mobility	R.S.D. (%)	Mobility	R.S.D. (%)
o-Cresol	−1.7701	0.556	−1.7964	0.381	−1.7401	0.695	−1.6608	2.313	−1.7871	0.629
Triphenylacetic acid[b]	−2.0496		−2.0496		−2.0496		−2.0496		−2.0496	
Probenecid	−2.1731	0.015	−2.1747	0.013	−2.1743	0.092	−2.1692	0.109	−2.1736	0.028
Ethacrynic acid	−2.2046	0.013	−2.2068	0.015	−2.2056	0.090	−2.2020	0.091	−2.2072	0.027
Diphenylacetic acid[b]	−2.4620		−2.4620		−2.4620		−2.4620		−2.4620	
Xanthene-9-carboxylic acid	−2.5493	0.012	−2.5494	0.013	−2.5500	0.065	−2.5459	0.099	−2.5492	0.015
Mandelic acid	−3.0568	0.015	−3.0555	0.017	−3.0593	0.090	−3.0576	0.055	−3.0557	0.036
Phenylacetic acid	−3.1367	0.014	−3.1365	0.013	−3.1379	0.084	−3.1374	0.064	−3.1366	0.065
Benzoic acid[b]	−3.4178		−3.4178		−3.4178		−3.4178		−3.4178	
meso-2,3-Diphenylsuccinic acid	−3.8967	0.038	−3.8915	0.022	−3.8962	0.301	−3.9048	0.063	−3.8924	0.070
1,2-Phenylenediacetic acid	−4.6412	0.034	−4.6371	0.058	−4.6345	0.361	−4.6483	0.094	−4.6315	0.100
o-Phthalic acid[b]	−5.0890		−5.0890		−5.0890		−5.0890		−5.0890	

[a]Mobilities expressed as 10^{-8} $m^2/(Vs)$.
[b]Marker compounds.

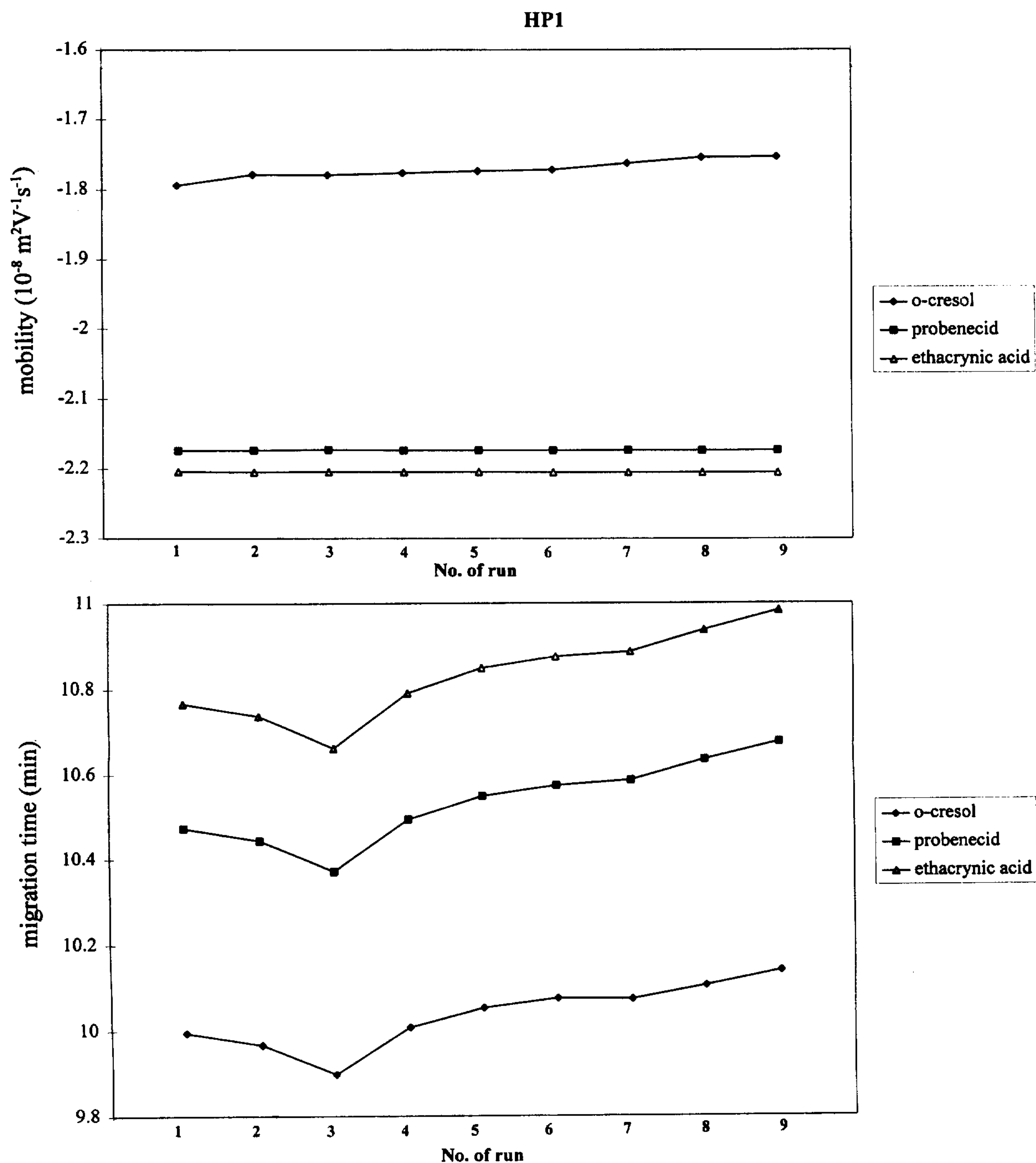

Fig. 2. Absolute migration times and electrophoretic mobilities for *o*-cresol, probenecid and ethacrynic acid for nine first runs in a fresh capillary. (this page) Data obtained with the HP1 instrument are shown, (next page) data obtained with the Waters Quanta 4000 instrument.

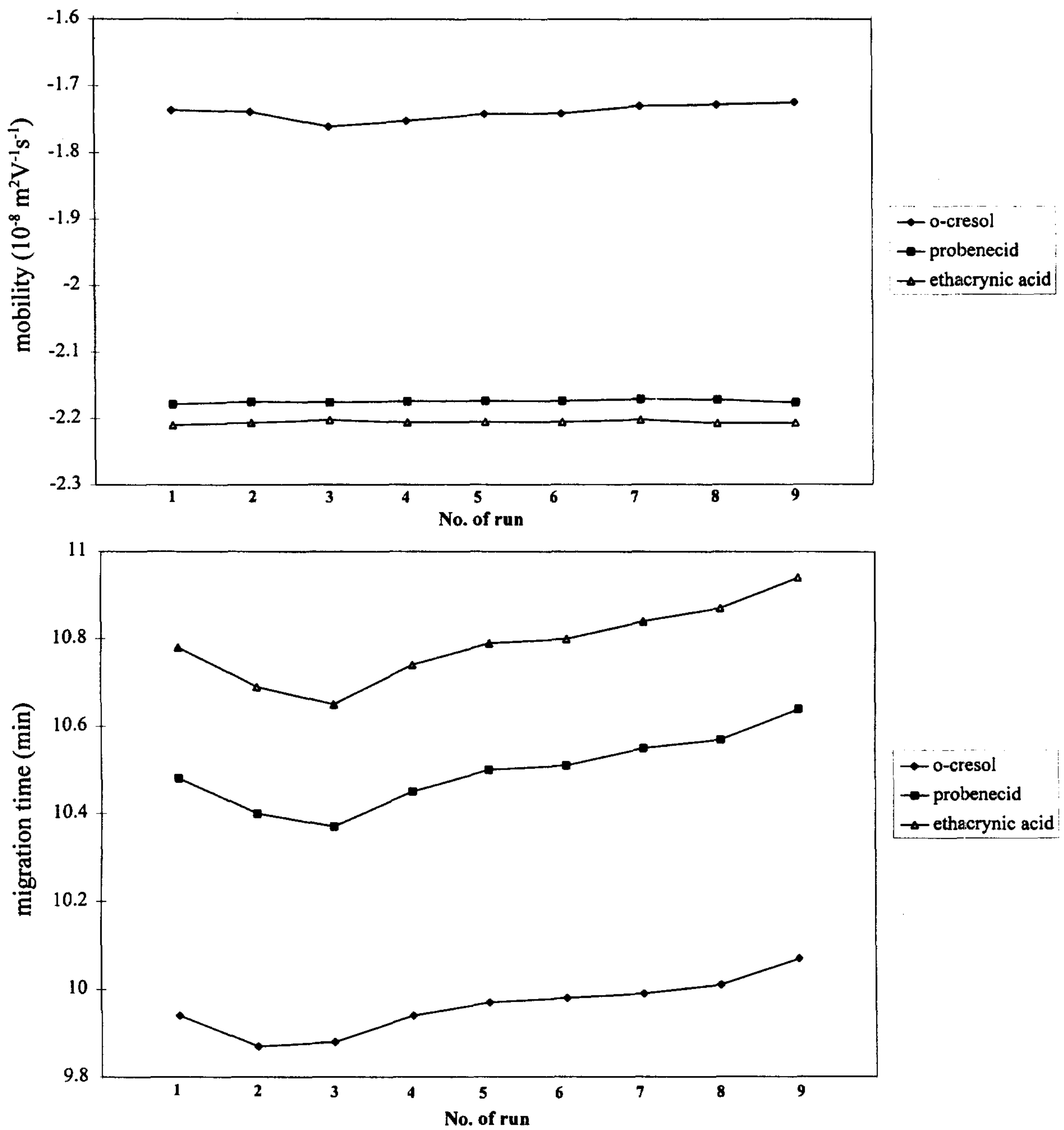

Fig. 2. (*continued*)

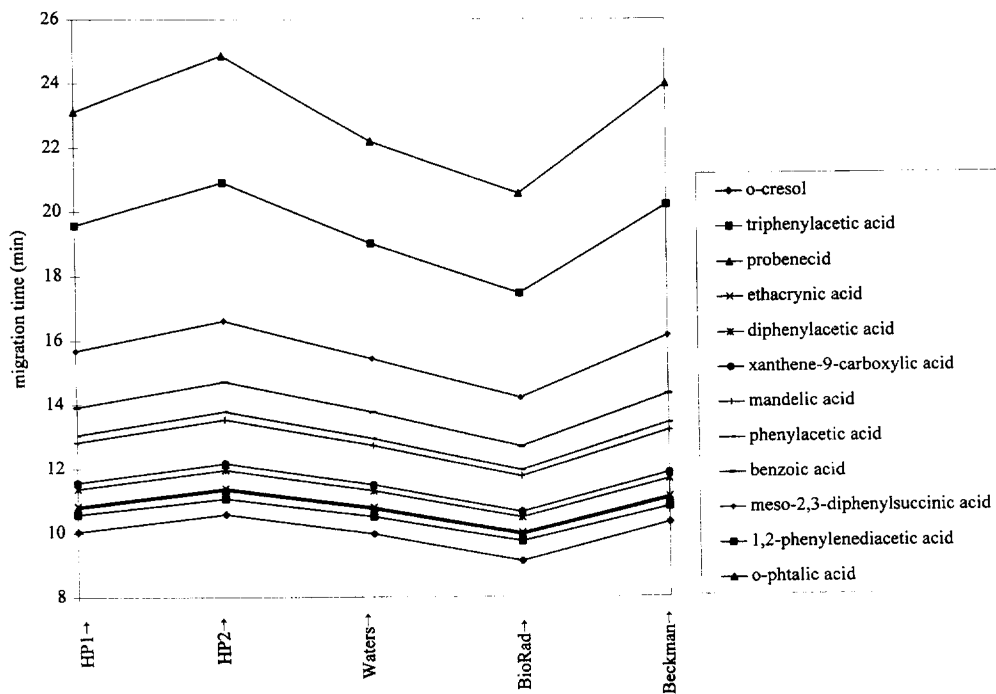

Fig. 3. Average migration times ($n=9$) of the analytes plotted against the different instruments.

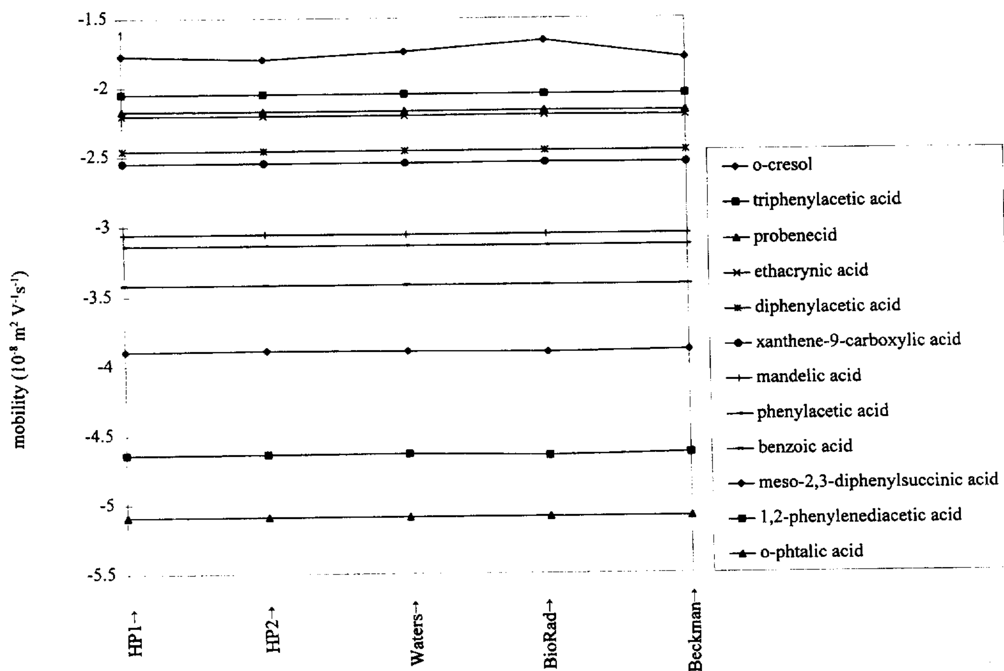

Fig. 4. Average mobilities ($n=9$) of the analytes plotted against the different instruments.

Table 3
Reproducibility of the migration times of the analytes measured with five different instruments, and average migration times and R.S.D. values for each analyte

Compound	HP1 Migr. time[a]	HP2 Migr. time	Waters Migr. time	BioRad Migr. time	Beckman Migr. time	Average of Migr. time	R.S.D. (%)
o-Cresol	10.03	10.58	9.96	9.11	10.31	10.00	5.54
Triphenylacetic acid[b]	10.57	11.06	10.50	9.73	10.80	10.53	4.76
Probenecid	10.77	11.32	10.73	9.94	11.04	10.76	4.83
Ethacrynic acid	10.83	11.39	10.79	9.99	11.11	10.82	4.85
Diphenylacetic acid[b]	11.37	11.96	11.31	10.47	11.66	11.35	4.91
Xanthene-9-carboxylic acid	11.56	12.17	11.49	10.63	11.86	11.54	4.97
Mandelic acid	12.83	13.53	12.72	11.75	13.17	12.80	5.21
Phenylacetic acid	13.05	13.78	12.94	11.95	13.41	13.03	5.28
Benzoic acid[b]	13.92	14.71	13.76	12.68	14.31	13.88	5.50
meso-2,3-Diphenylsuccinic acid	15.68	16.61	15.43	14.20	16.14	15.61	5.84
1,2-Phenylenediacetic acid	19.57	20.89	19.00	17.44	20.18	19.42	6.75
o-Phthalic acid[b]	23.11	24.86	22.18	20.53	23.96	22.93	7.28

[a]Migration times in minutes ($n=9$).
[b]Marker compounds.

values for *o*-cresol were higher, between 0.38 and 2.32%, due to the pK_a value of *o*-cresol being close to the pH of the buffer.

In all instruments, the migration times of the components decreased at first, and then, from the fourth run on, they increased slightly. This means that, when the capillary was conditioned for 5 min each with potassium hydroxide and water, the system was stabilized after three runs. However, when mobilities calculated with the marker technique were used, run-to-run differences in the values were avoided. Fig. 2 illustrates this for the three first-migrating analytes for the HP1 and Waters Quanta instruments. The small overall decrease in the mobility of *o*-cresol is probably due to changes in pH caused by electrode reactions [13].

A plot of the average migration times of the analytes ($n=9$) (Fig. 3) shows that there were wide differences in the migration times for different instruments. The corresponding plot of the average

Table 4
Reproducibility of the mobilities of the analytes, obtained with the 4m technique and five different instruments, and average mobilities and R.S.D. values for each analyte

Compound	HP1 mobility[a]	HP2 mobility	Waters mobility	BioRad mobility	Beckman mobility	Average mobility	R.S.D. (%)
o-Cresol	−1.7701	−1.7964	−1.7401	−1.6608	−1.7871	−1.7509	3.123
Triphenylacetic acid[b]	−2.0496	−2.0496	−2.0496	−2.0496	−2.0496	−2.0496	
Probenecid	−2.1731	−2.1747	−2.1743	−2.1692	−2.1736	−2.1730	0.101
Ethacrynic acid	−2.2046	−2.2068	−2.2056	−2.2020	−2.2072	−2.2052	0.094
Diphenylacetic acid[b]	−2.4620	−2.4620	−2.4620	−2.4620	−2.4620	−2.4620	
Xanthene-9-carboxylic acid	−2.5493	−2.5494	−2.5500	−2.5459	−2.5492	−2.5488	0.064
Mandelic acid	−3.0568	−3.0555	−3.0593	−3.0576	−3.0557	−3.0570	0.051
Phenylacetic acid	−3.1367	−3.1365	−3.1379	−3.1374	−3.1366	−3.1370	0.019
Benzoic acid[b]	−3.4178	−3.4178	−3.4178	−3.4178	−3.4178	−3.4178	
meso-2,3-Diphenylsuccinic acid	−3.8967	−3.8915	−3.8962	−3.9048	−3.8924	−3.8963	0.135
1,2-Phenylenediacetic acid	−4.6412	−4.6371	−4.6345	−4.6483	−4.6315	−4.6385	0.141
o-Phthalic acid[b]	−5.0890	−5.0890	−5.0890	−5.0890	−5.0890	−5.0890	

[a]Mobilities expressed as 10^{-8} $m^2/(Vs)$.
[b]Marker compounds.

mobilities of the analytes (Fig. 4) instead shows fluctuations in the mobilities to be rather small, regardless of the type of injection and the cooling system. No large variations in mobilities between instruments due to unthermostated segments of the capillaries were observed.

Table 3 Table 4 present the data on the reproducibility of the migration times and mobilities. Except for *o*-cresol, the reproducibility of the mobilities was very good: the R.S.D. values for the mobilities were between 0.06 and 0.65%. The corresponding values for the reproducibility of the absolute migration times were about 5%. Accordingly, when a specific optimized analysis is transferred to another instrument, it will work well when markers are used and the pK_a values of the analytes differ sufficiently from the pH of the buffer solution. Since the buffer was always fresh, prepared the same day by the standard procedure, and since a new capillary was used in all instruments, the study shows that the mobilities calculated with the marker techniques are not only highly repeatable run-to-run but also highly reproducible for different instruments.

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