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# Separation of aromatic sulphonic acid dye intermediates by high-performance liquid chromatography and capillary zone electrophoresis

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## Abstract

The possibilities of separating various aromatic sulphonic acids used as intermediates in production of synthetic dyes by reversed-phase high-performance liquid chromatography (HPLC) and capillary zone electrophoresis (CZE) were studied. Isomeric naphthalene mono- to tetrasulphonic acids are best separated by HPLC with aqueous–methanolic mobile phases containing sodium sulphate. A high content of bonded hydrocarbon moieties is essential for successful separation. Components of technological samples of 4,4'-dinitrostilbene-2,2'-disulphonic acid can also be successfully separated using this technique, but the separation is very sensitive to the concentration of methanol in the mobile phase. Various aromatic aminosulphonic acids (letter acids) in technological samples can be separated by ion-pair reversed-phase chromatography with mobile phases containing tetrabutylammonium ions, but a better separation is possible using capillary zone electrophoresis with working electrolytes containing  $\beta$ -cyclodextrin. The opposite applies to the separation of naphthalenesulphonic acids and dinitrostilbenedisulphonic acid isomers, so that the HPLC and CZE are complementary to each other. Both HPLC and CZE techniques are suitable for the quantitative analysis of technical products containing aromatic sulphonic acids.

*Keywords:* Mobile phase composition; Sulfonic acids; Aromatic compounds

## 1. Introduction

Aromatic sulphonic acids are important intermediates in the production of a variety of synthetic dyes, optical brighteners and fluorescent whitening agents. High-performance liquid chromatography (HPLC) and capillary zone electrophoresis (CZE) are well suited for the separation of these anionic compounds.

Liquid–liquid chromatography with *n*-octyl-

amine stationary phase was attempted for the separation of aromatic sulphonic acids, but a long time is necessary to attain equilibrium between the adsorbent, the liquid stationary phase and the mobile phase [1]. Anion-exchange chromatography has occasionally been used for their separation [2–6], but this method usually lacks selectivity for the separation of isomeric compounds. In reversed-phase systems with pure aqueous–organic mobile phases the acids are usually eluted close to the column dead volume with little separation and often even with strong-

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ly asymmetric peak shapes [7]. To increase the retention and to achieve successful separations, it is necessary to add ionic compounds to the mobile phase.

Sulphonic acids are strong acids, completely dissociated in aqueous or aqueous–organic solutions over a broad pH range, so that diluted acidic buffers cannot be used to suppress their ionization and improve their chromatographic properties. Aromatic aminosulphonic acids are an exception to this rule and their acid–basic properties are similar to those of carboxylic acids. Consequently, separation of these acids in buffered aqueous organic mobile phases is in principle possible [8]. This is not the case with other aromatic sulphonic acids, which can be separated using ion-pair reversed-phase chromatography with mobile phases containing tetraalkylammonium salts at concentrations of  $10^{-3}$ – $10^{-4}$  mol/l [9–12].

The retention and the separation selectivity depend on the nature of the ion-pairing reagent, on the number of sulphonic groups, on the character of the aromatic ring and on the nature, number and position of the substituents. The retention can be controlled by adjusting the concentration of both the ion-pairing reagent and the organic solvent in the mobile phase [13–15]. This technique has been used successfully to separate some unsubstituted naphthalene- and anthraquinone sulphonic acids [13,16] and their hydroxy and amino derivatives (letter acids) [17–19]. However, it failed to resolve complex mixtures containing various isomers of naphthalenesulphonic acids with different numbers of sulphonic groups [13].

The separation of sulphonic acids can also be achieved by reversed-phase HPLC with mobile phases containing strong electrolytes (salts) at concentrations of 0.1–1 mol/l. In the presence of salts ionic interactions in the stationary phase are suppressed, retention is increased and the separation selectivity is enhanced [7,20]. The retention can be controlled by adjusting the concentrations of either the salt or the organic solvent in mixed mobile phases. This technique makes it possible to separate not only 1- and 2-naphthalenesulphonic acids [21,22], but also more than ten isomeric naphthalene mono- to

tetrasulphonic acids [23,24], which are eluted in the order of decreasing number of sulphonic groups. Substituted amino- and hydroxy-naphthalenesulphonic acids [20,25] or isomers of 4,4'-diaminostilbene-2,2'-disulphonic acid [26] can also be separated. Aqueous–organic mobile phases have been used to separate sulphonic acid derivatives of phenol on a  $\beta$ -cyclodextrin bonded phase [27].

Although CZE seems ideally suited for the analysis of aromatic sulphonic acids, little work in this field has been published so far. In addition to electrophoretic separations of acidic azo dyes in industrial products [28–30] and in the environment [31], CZE has been applied to the separation of several substituted benzenesulphonic [32] and anthraquinonesulphonic [16] acids and isomeric 4,4'-diaminostilbene-2,2'-disulphonic acid [26]. Some acidic azo dyes and aromatic sulphonic acids could be separated by micellar electrokinetic capillary chromatography in borate buffers containing cholic acid [33].

A few earlier results [16,29] indicate that HPLC and capillary electrophoresis complement each other with respect to the resolution of aromatic sulphonic acids. The purpose of this work was to compare the possibilities of separating various acids used as intermediates in the dye and textile industry using the two instrumental techniques:

(1) twelve naphthalenesulphonic acids differing in number and position of the sulphonic groups, occurring in reaction mixtures after sulphonation of naphthalene;

(2) technological samples of the *E* isomer of 4,4'-dinitrostilbene-2,2'-disulphonic acid (DNSA), which may contain the *Z* isomer formed by photochemical rearrangement on exposure to the light and 4,4'-dinitrobenzyl-2,2'-disulphonic acid (DNBA) as a by-product, in analogy with the samples of technical 4,4'-diaminostilbene-2,2'-sulphonic acid produced by acid reduction of DNSA [26];

(3) five acids occurring in technological samples of Cleve acids (Laurent, amino-F-, Cleve-1,6-, peri and Cleve-1,7-);

(4) technological samples of 1-(2',5'-dichloro-4'-sulpho)phenyl-3-methyl-5-pyrazolone (DCSPMP) containing 2,5-dichloro-4-sul-

phophenylhydrazine (DCSPH) and 2,5-dichloroaniline-4-sulphonic acid (DCASA);

(5) technological samples of H-acid containing chromotropic, Koch and W acids; and

(6) technological samples of I-acid containing  $\gamma$ -, amino-I-, di-I-, amino-F- and dioxy-I-acids.

## 2. Experimental

### 2.1. Chemicals

Pure standards and technical samples of sulphonic acids were obtained from Synthesia (Pardubice-Semtín, Czech Republic). Their structures are given in Table 1. Individual standards, synthetic mixtures and real samples were dissolved at appropriate concentrations in the mobile phases or in the working electrolytes used.

Methanol (spectroscopic grade), sodium tetraborate, boric acid, sodium dihydrogenphosphate, disodium monohydrogenphosphate, sodium sulphate and sodium hydroxide (all analytical-reagent grade) were obtained from Lachema (Brno, Czech Republic). Tetrabutylammonium hydrogensulphate (analytical grade) was purchased from Merck (Darmstadt, Germany) and  $\beta$ -cyclodextrin (analytical-reagent grade) from Serva (Heidelberg, Germany). Deionized water was doubly distilled in glass with addition of potassium permanganate. Mobile phases for HPLC were prepared by dissolving the required quantities of tetrabutylammonium hydrogensulphate or sodium sulphate in water or in aqueous methanol. Working electrolytes for CZE were prepared by dissolving the buffer components in water. The pH was adjusted by adding a few drops of sodium hydroxide using an OP 208 pH meter (Radelkis, Budapest, Hungary). All mobile phases and electrolytes were filtered using a Millipore 0.45- $\mu$ m filter and degassed by ultrasonication before use.

### 2.2. High-performance liquid chromatography

An HP 1090M liquid chromatograph equipped with a diode-array UV detector operated at 230 nm, an automatic sample injector, a DR5 solvent-delivery system, a thermostated column

compartment and a Series 7994A workstation was used for the HPLC experiments. The following columns were used: (1) 150  $\times$  3.3 mm I.D., glass cartridge, Separon SGX RPS, 7  $\mu$ m (Tessek, Prague, Czech Republic); (2) 250  $\times$  4.0 mm I.D., stainless steel, Separon SGX RPS, 7  $\mu$ m (Tessek); (3) 150  $\times$  3.3 mm I.D., glass cartridge, Separon SGX C<sub>18</sub>, 7  $\mu$ m (Tessek); (4) 250  $\times$  4.0 mm I.D., stainless steel, Separon RPS-1, 10  $\mu$ m (Tessek); (5) 300  $\times$  3.6 mm I.D., stainless steel, packed in the laboratory with Silasorb SPH C<sub>18</sub>, 7.5  $\mu$ m (Lachema); (6) 300  $\times$  4.2 mm I.D., stainless steel, packed in the laboratory with Silasorb SPH C<sub>8</sub>, 7.5  $\mu$ m (Lachema); and (7) 300  $\times$  3.6 mm I.D., stainless steel, packed in the laboratory with LiChrosorb RP-18, 7  $\mu$ m (Merck).

The column temperature was set at 40°C, the flow-rate at 1 ml/min and the detection wavelength at 230 nm. The mobile phase in the solvent reservoir was continuously stripped by a stream of helium during the run. Sample volumes of 5–25  $\mu$ l were injected.

The column dead volume,  $V_M$ , was determined using methanol as the dead volume marker and refractometric detection with an R 401 differential refractometer (Waters–Millipore).

The retention volumes,  $V_R$ , of the standards were measured at different mobile phase compositions. The mean values of  $V_R$  from three repeated experiments were used for calculations of the capacity factors,  $k' = (V_R/V_M - 1)$ , of the individual sulphonic acids.

### 2.3. Capillary zone electrophoresis

A Crystal 310 instrument (ATI Unicam, Cambridge, UK) equipped with a variable-wavelength detector was used for all CZE measurements. Silica capillaries, 75 cm (60 cm effective length to the detector)  $\times$  50 or 75  $\mu$ m I.D., were subsequently washed with 0.1 mol/l NaOH (10 min), water (10 min) and working electrolyte (until a stabilized baseline was obtained, ca. 20 min) before use. The temperature of the capillary was set at 30°C and the samples were introduced into the capillary using pressurized injection (0.2–0.5 min at 25 mbar). The separation was performed at a potential of +25 kV applied

Table 1  
Compounds analysed, structure and abbreviations used in the text and figures

1-naphthalene sulphonic acid (1-NSA)	2-naphthalene sulphonic acid (2-NSA)	1-aminonaphthalene-6-sulphonic acid (Cleve-1,6-acid)	1-aminonaphthalene-7-sulphonic acid (Cleve-1,7-acid)
1,3-naphthalene disulphonic acid (1,3-NDSA)	1,5-naphthalene disulphonic acid (1,5-NDSA)	5-aminonaphthalene-1-sulphonic acid (Laurent acid)	8-aminonaphthalene-1-sulphonic acid (peri acid)
1,6-naphthalene disulphonic acid (1,6-NDSA)	1,7-naphthalene disulphonic acid (1,7-NDSA)	7-amino-1-hydroxynaphthalene-3-sulphonic acid ( $\gamma$ -acid)	2-aminonaphthalene-7-sulphonic acid (amino-F-acid)
2,6-naphthalene disulphonic acid (2,6-NDSA)	2,7-naphthalene disulphonic acid (2,7-NDSA)	6-amino-1-hydroxynaphthalene-3-sulphonic acid (I-acid)	6-aminonaphthalene-1,3-disulphonic acid (amino-I-acid)
1,3,5-naphthalene trisulphonic acid (1,3,5-NTSA)	1,3,6-naphthalene trisulphonic acid (1,3,6-NTSA)	1,6-dihydroxynaphthalene-3-sulphonic acid (dioxyl-acid)	1-amino-8-hydroxynaphthalene-3,6-disulphonic acid (H-acid)
1,3,7-naphthalene trisulphonic acid (1,3,7-NTSA)	1,3,5,7-naphthalene tetrasulphonic acid (1,3,5,7-NTeSA)	8-aminonaphthalene-1,3,6-trisulphonic acid (Koch acid)	8-amino-3-hydroxynaphthalene-1,6-disulphonic acid (W-acid)
Z-4,4'-dinitrostilbene-2,2'-disulphonic acid (Z-DNSA)	E-4,4'-dinitrostilbene-2,2'-disulphonic acid (E-DNSA)	1,8-dihydroxynaphthalene-3,6-disulphonic acid (chromotropic acid)	2,5-dichloro-4-sulphophenylhydrazine (DCSPH)
4,4'-dinitrostilbene-2,2'-disulphonic acid (DNBA)		2,5-dichloroaniline-4-sulphonic acid (DCASA)	5,5'-dihydroxy-2,2'-dinaphthylamine-7,7'-disulphonic acid (di-I-acid)
		1-(2',5'-dichloro-4'-sulpho)-phenyl-3-methyl-pyrazolone (DCSPMP)	

across the capillary. The detection wavelength was set at 230 nm, as in the HPLC experiments.

### 3. Results and discussion

#### 3.1. High-performance liquid chromatography

We found that the separation of the isometric tri- and tetrasulphonic acids is extremely sensitive to the non-polar column packing material used (chromatograms A–F in Fig. 1). The separation improves as the amount of the bonded carbon moiety increases – the four acids tested are co-eluted in a single peak on a C<sub>8</sub> bonded phase (A), while partial separation can be achieved on common C<sub>18</sub> columns (B–D) and

the best separation is accomplished on columns with a higher density of the octadecyl-bonded phase (RPS type with carbon contents >22%, E and F) and better shielding of the residual silanol groups. The differences between the separation on the columns of the same type (B–D and E, F) are far less significant and can be at least partly attributed to the different particle sizes of the materials and geometries of the columns. The best resolution (E) was achieved on the Separon SGX RPS column, which was used to separate the mixture of two mono-, six di-, three tri- and one naphthalenetetrasulphonic acids most likely to occur in reaction mixtures after sulphonation of naphthalene. The acids were eluted in order of decreasing number of sulphonic groups using isocratic elution with 0.4 mol/l Na<sub>2</sub>SO<sub>4</sub> in water

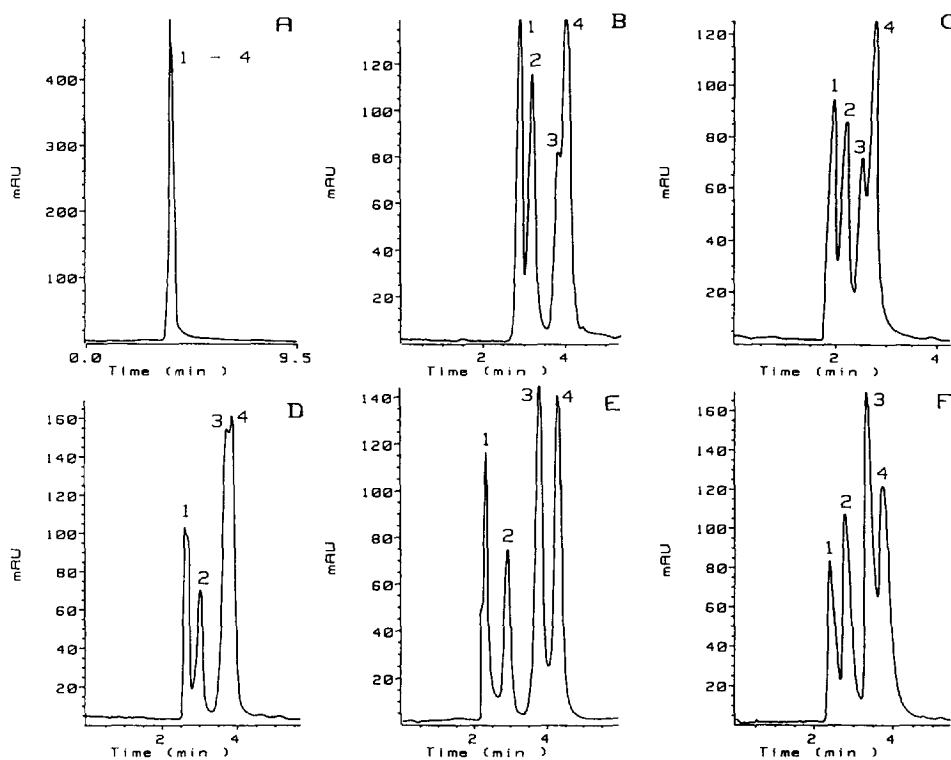


Fig. 1. Comparison of HPLC separation of 1,3,5,7-NTeSA (1), 1,3,6-NTSA (2), 1,3,5-NTSA (3) and 1,3,7-NTSA (4) on columns with different packing materials. Columns: (A) Silasorb SPH C<sub>8</sub>, 7.5 μm, 300 × 4.2 mm I.D.; (B) Silasorb SPH C<sub>18</sub>, 7.5 μm, 300 × 3.6 mm I.D.; (C) Separon SGX C<sub>18</sub>, 7 μm, 150 × 3 mm I.D.; (D) LiChrosorb RP-18, 7 μm, 300 × 3.6 mm I.D.; (E) Separon SGX RPS, 7 μm, 250 × 4 mm I.D.; (F) Separon SGX RPS-1, 10 μm, 250 × 4 mm I.D. Mobile phase, 0.4 mol/l Na<sub>2</sub>SO<sub>4</sub>; flow-rate, 1 ml/min, except for chromatogram C (0.5 ml/min); column temperature, 40°C; detection, UV at 230 nm.

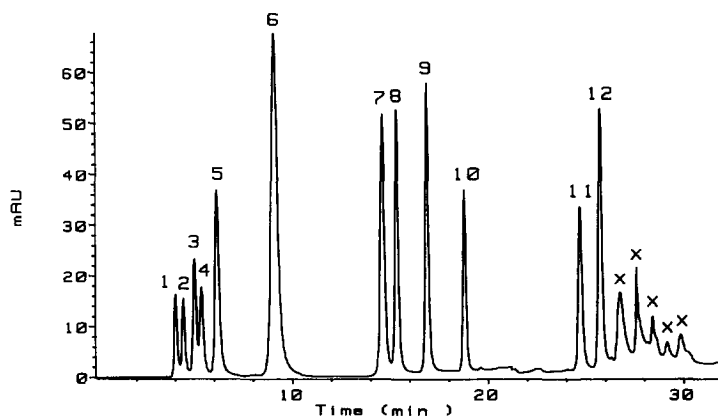


Fig. 2. HPLC separation of a mixture of twelve naphthalenesulphonic acids. Peaks: 1 = 1,3,5,7-NTSA; 2 = 1,3,6-NTSA; 3 = 1,3,5-NTSA; 4 = 1,3,7-NTSA; 5 = 1,5-NDSA; 6 = 2,6-NDSA; 7 = 1,6-NDSA; 8 = 2,7-NDSA; 9 = 1,3-NDSA; 10 = 1,7-NDSA; 11 = 1-NSA; 12 = 2-NSA; X = unidentified less polar impurities. Column, Separon SGX RPS, 7  $\mu$ m, 250  $\times$  4 mm I.D.; solvent programme, 5 min isocratic, 0.4 mol/l  $\text{Na}_2\text{SO}_4$  at 0.5 ml/min, followed by a linear gradient from 0.4 mol/l  $\text{Na}_2\text{SO}_4$  to 40% (v/v) methanol in water in 15 min at 1 ml/min; column temperature, 40°C; detection, UV at 230 nm.

for the first 5 min, followed by a ternary linear gradient from 0.4 to 0 mol/l  $\text{Na}_2\text{SO}_4$  and from 0 to 40% aqueous methanol in 15 min (Fig. 2).

These separation conditions can be used for the determination of the individual sample components. The calibration graphs were linear in the range 0.05–1 g/l and the relative error of three repeated experiments was 1–4%.

Samples of technical *E* isomer of DNSA, containing the *Z* isomer and DNBA, can be separated in mobile phases with 0.3 mol/l sodium sulphate on both Separon SGX  $\text{C}_{18}$  and Separon SGX RPS columns. The separation is significantly affected by the concentration of methanol in the mobile phase. The selectivity of separation of the *Z* isomer from DNBA is insufficient at low concentrations of methanol, in addition to the elution times being too long. At high concentrations of methanol the selectivity of separation of the *E* and *Z* isomers of DNSA and the retention are too low for a good separation. The best separation can be achieved on the Separon SGX  $\text{C}_{18}$  column with a mobile phase containing 25% methanol. Under these conditions, good linearities of the calibration graphs of the *E* isomer of DNSA and of DNBA with correlation coefficients of 0.99998 and 0.99999 were found at the detection wavelength of 230 nm in the concentration range 0.02–0.2 g/l.

Some aminonaphthalene sulphonic acids can also be resolved using mobile phases with sodium sulphate, such as a mixture containing Cleve-1,6-, Cleve-1,7-, Laurent and peri acids with a mobile phase containing 0.4 mol/l  $\text{Na}_2\text{SO}_4$  in 1.5% methanol. Other acids are little retained even in mobile phases containing 0.4 mol/l  $\text{Na}_2\text{SO}_4$  in water and cannot be separated using this methods.

The capacity factors of aromatic aminosulphonic acids on a Separon SGX RPS column with mobile phase containing 0.005 mol/l tetrabutylammonium hydrogensulphate as ion-pairing reagent with various concentrations of methanol are given in Table 2. An almost linear decrease in  $\log k$  is observed as the concentration of methanol is increased and the slopes of the plots are close to each other for the different acids. With mobile phases containing 15% methanol, it is possible to separate five acids occurring in technical Cleve acid in 10 min. The acids elute in the order Laurent, amino-F-, Cleve-1,6-, peri and Cleve-1,7- (Fig. 3).

The resolution of all important components potentially present in technological samples in the chromatogram in Fig. 3 is sufficient for quantitative analysis. At the detection wavelength of 230 nm the calibration graphs for the acids are linear in the concentration range

Table 2  
Capacity factor ( $k'$ ) of the aromatic sulphonic acids on a Separon SGX RPS ( $7 \mu\text{m}$ ) column ( $150 \times 3 \text{ mm}$  I.D.) with mobile phase containing  $0.005 \text{ mol/l}$  tetrabutylammonium hydrogensulphate and different concentrations of methanol at  $40^\circ\text{C}$

Compound	Methanol concentration (%)						
	3	6	9	12	15	21	30
$\gamma$ -Acid	4.99	2.57	1.65	1.16			
I-Acid	5.39	3.27	1.96	1.42			
Amino-F-acid	10.9	7.09	3.72	2.63	2.25	1.10	
Cleve-1,6-acid		12.0			3.76	1.95	
Cleve-1,7-acid		39.9			11.6	5.64	
Peri acid		14.3			4.66	2.41	
Laurent acid		3.38			1.19	0.62	
DCSPMP					28.0	12.1	4.31
DCSPH					1.30	0.69	0.31
DCASA					15.6	7.46	2.94

$0.0002$ – $0.01 \text{ g/l}$  with limits of determination of  $0.00012$ – $0.00024 \text{ g/l}$ . The correlation coefficients were in the range  $0.9991$ – $0.99997$ .

With the mobile phase containing  $0.005 \text{ mol/l}$  tetrabutylammonium hydrogensulphate in  $30\%$  methanol, technological samples containing DCSPH, DCASA and DCSPMP are completely resolved in less than  $5 \text{ min}$ . It was possible to separate H-acid and chromotropic acid from each other, or amino-F- acid from  $\gamma$ - and I-acids,

but all the acids potentially present in technological samples of I-acid and of H-acid were not completely separated in the ion-pair systems tested.

### 3.2. Capillary zone electrophoresis

Because the electroosmotic migration velocity is higher than the electrophoretic migration velocity of the acids tested, a positive potential should be applied across the capillary and the acids should migrate from the source to the detector at a velocity lower than that of the electroosmotic flow. The selectivity of separation does not depend significantly on the applied potential, but the migration times decrease as the potential is increased. To keep the time of analysis short, the separations were run at  $25 \text{ kV}$ . An increase to  $30 \text{ kV}$  did not bring about a significant decrease in migration times.

Naphthalenesulphonic acids showed two broad bands in  $0.025 \text{ mol/l}$  borate buffer and in  $0.025 \text{ mol/l}$  phosphate buffer ( $\text{pH } 8.0$ ) were separated into four peaks according to the increase in number of sulphonic groups. The separation of the individual isomers co-eluted in the four peaks was not possible at any phosphate concentration or  $\text{pH}$ .

Pure and mixed  $0.025 \text{ mol/l}$  borate and phosphate buffers were tested as working electrolytes of the separation of various aromatic amino-

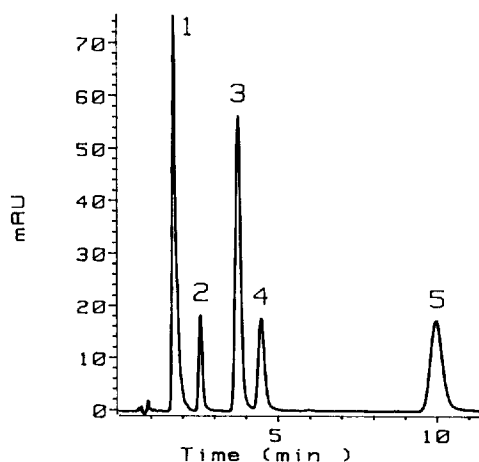


Fig. 3. HPLC separation of (1) Laurent, (2) amino-F-, (3) Cleve-1,6-, (4) peri and (5) Cleve-1,7-acids. Column, Separon SGX RPS,  $7 \mu\text{m}$ ,  $150 \times 3 \text{ mm}$  I.D.; mobile phase,  $0.005 \text{ mol/l}$  tetrabutylammonium hydrogensulphate in  $15\%$  ( $v/v$ ) methanol in water; flow-rate,  $0.5 \text{ ml/min}$ ; column temperature,  $40^\circ\text{C}$ ; detection, UV at  $230 \text{ nm}$ .

sulphonic acids. The shapes of the peaks were best in the 1:1 borate–phosphate buffer (0.025 mol/l, pH 8.0), where it was possible to achieve the separation of the  $\gamma$ - or I-acids from Cleve acids and to separate partially Cleve-1,6- and Cleve-1,7-acids, but other acids tested remained unresolved, like the isomeric naphthalenesulphonic acids and DNSA isomers from DNBA.

We investigated the possibilities of improving the separation by addition of  $\beta$ -cyclodextrin to the working electrolyte. This additive is a well known chiral selector widely used for CZE separations of optical isomers. Because of the different accessibility of the cavity in its ring-shaped molecule, it can also enhance the resolution of various positional isomers.  $\beta$ -Cyclo-

dextrin can form inclusion complexes with compounds of suitable size and shape. These solutes migrate only for part of the time in the electrolyte as free ions and for the remaining time they move together with cyclodextrin, the migration velocity of which is controlled only by the electroosmotic flow. This effect can accelerate the migration of negatively charged solutes to the detector and decrease their time of migration. Different stabilities of the inclusion complexes of various sample components may result in significant differences between their migration times.

The separation of various sulphonic acids improved significantly on using the working electrolyte containing  $\beta$ -cyclodextrin. In 0.025 mol/l phosphate buffer (pH 8) containing 0.01 mol/l  $\beta$ -cyclodextrin it was possible to accom-

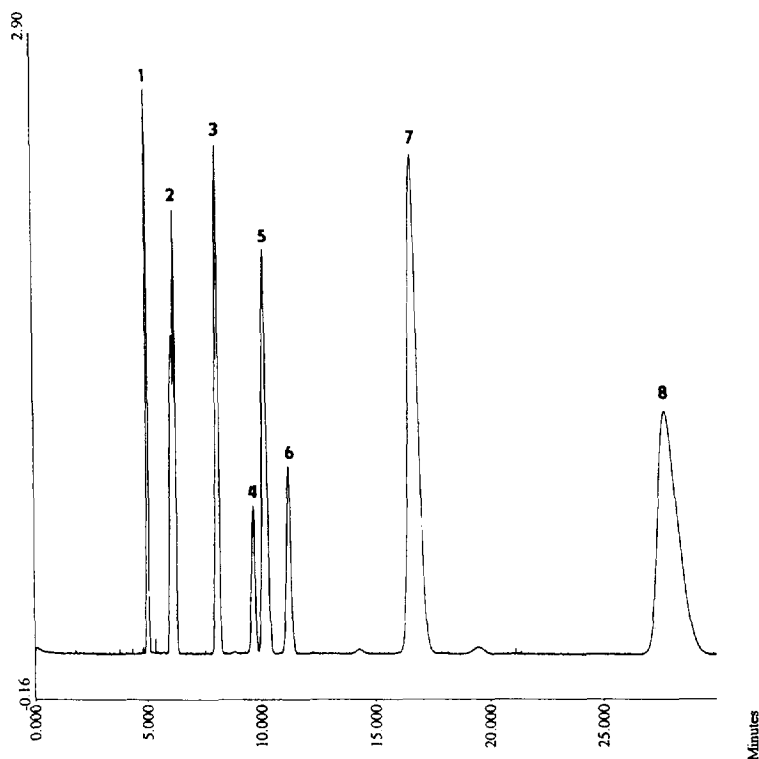


Fig. 4. CZE separation of a mixture of naphthalenesulphonic acids. Peaks: 1 = 2-NSA; 2 = 1-NSA, 2,6-NDSA and 2,7-NDSA; 3 = 1,6-NDSA; 4 = 1,3-NDSA; 5 = 1,5-NDSA and 1,7-NDSA; 6 = 1,3,6-NTSA; 7 = 1,3,5-NTSA and 1,3,7-NTSA; 8 = 1,3,5,7-NTeSA. Capillary, 75 cm (60 cm to detector)  $\times$  75  $\mu$ m I.D. uncoated fused silica; 0.025 mol/l phosphate buffer (pH 9) + 0.01 mol/l  $\beta$ -cyclodextrin; voltage, +25 kV; injection at 25 mbar for 0.2 min; pressure, 25 mbar applied across capillary during analysis; capillary temperature, 30°C; detection, UV at 230 nm.



plish the separation of some individual isomers of naphthalene mono- to tetra sulphonic acids under a pressure of 25 mbar applied across the capillary (Fig. 4). However, the pairs of isomeric 2,6-, 2,7- and 1,5-, 1,7-naphthalenedisulphonic acids and 1,3,5- and 1,3,7-naphthalenetrisulphonic acids remained unresolved and the separation of 1-naphthalenesulphonic from 2,6- and 2,7-naphthalenedisulphonic acid was poor.

In mixed 0.025 mol/l phosphate–borate buffer containing 0.01 mg/l  $\beta$ -cyclodextrin, complete separation was achieved (1) of DCSPH, DCASA and DCSPMP, (2) of amino-F-, Cleve-1,6-, Cleve-1,7-, Laurent and peri acids (Fig. 5) and (3) of the two Cleve acids from  $\gamma$ - and I-acids. Under these conditions, the separation of chromotropic acid from H-acid is incomplete.

The effective mobilities of weak amino arylsulphonic acids depend on the pH of the working

electrolyte. In 0.025 mol/l mixed phosphate–borate buffer (pH 8.5) containing 0.01 mol/l  $\beta$ -cyclodextrin, complete separation of chromotropic, H-, W- and Koch acids was accomplished in 17 min under increased pressure (Fig. 6).

Sulphonic acids occurring in technological samples of I-acid could not be resolved successfully in mixed phosphate–borate buffers at any pH in the range 7–9, but in 0.025 mol/l borate buffer (pH 9) containing 0.01 mol/l  $\beta$ -cyclodextrin all the acids (amino-F-, I-,  $\gamma$ -, dioxy-I-, amino-I- and di-I-) are successfully separated in 17 min (Fig. 7).

Under the conditions providing optimum separation, the samples can be analysed quantitatively. Table 3 gives the results of their statistical evaluation. The calibration graphs are linear in the range 0.5–100 mg/l. Figs. 8 and 9 show example of the separation of the individual acids

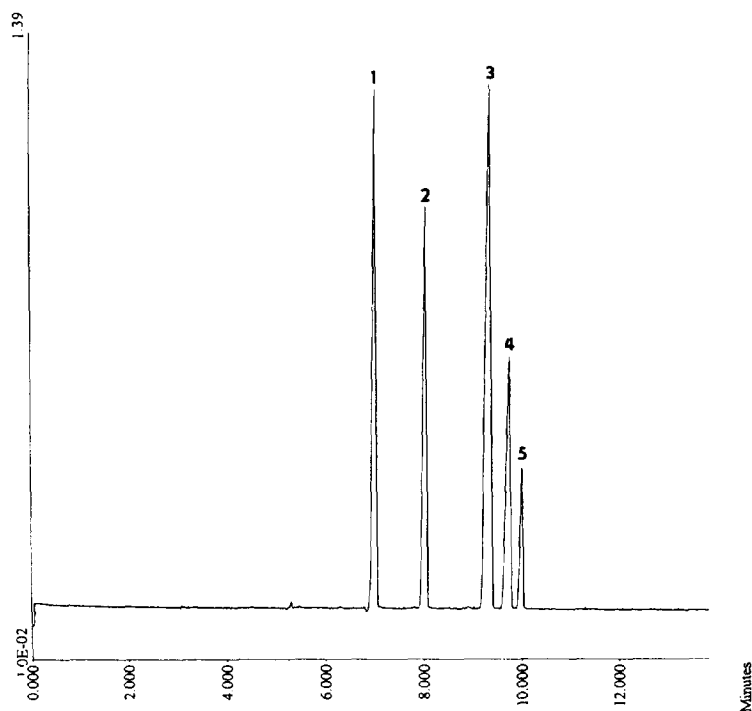


Fig. 5. CZE separation of (1) amino-F-, (2) Cleve-1,6-, (3) Cleve-1,7-, (4) Laurent and (5) peri acids. Capillary: 75 cm (60 cm to detector)  $\times$  50  $\mu$ m I.D. uncoated fused silica; buffer, 0.025 mol/l phosphate–borate (1:1) (pH 8) + 0.01 mol/l  $\beta$ -cyclodextrin; voltage, +25 kV; injection at 25 mbar for 0.2 min; capillary temperature, 30°C; detection, UV at 230 nm.

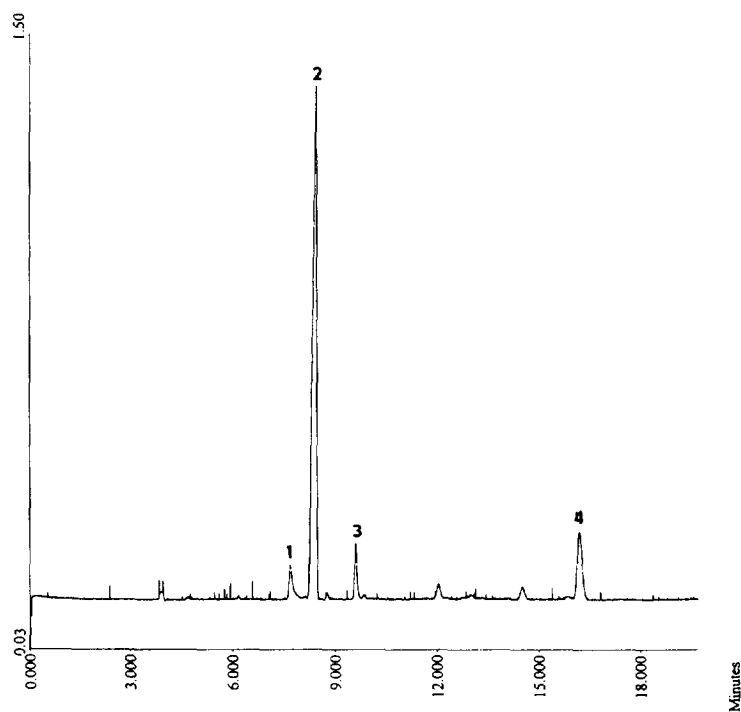


Fig. 6. CZE separation of (1) chromotropic, (2) H-, (3) W- and (4) Koch acids. Conditions as in Fig. 5 except for the pH of the working buffer, adjusted to 8.5, and injection at 25 mbar for 0.5 min.

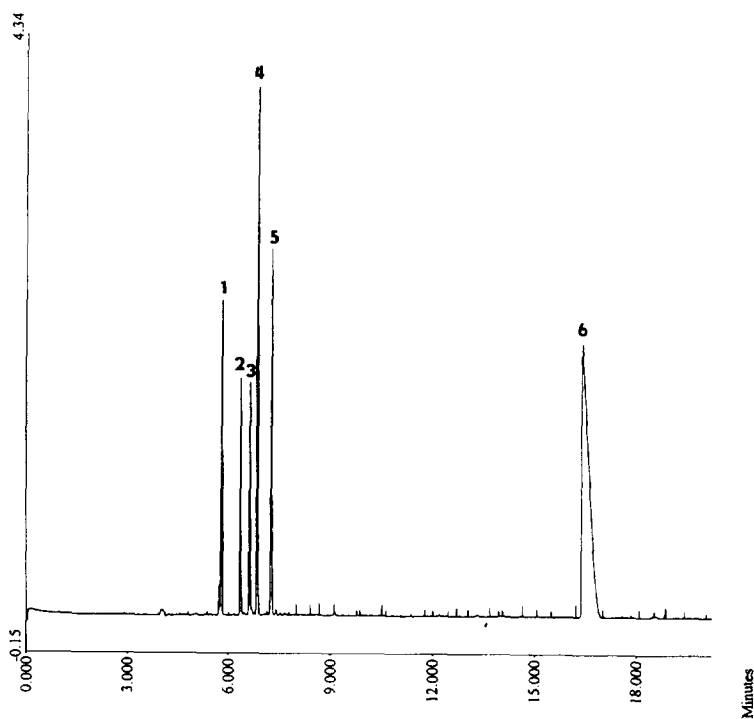


Fig. 7. CZE separation of (1) amino-F-, (2) I-, (3)  $\gamma$ -, (4) dioxy-I-, (5) amino-F- and (6) di-I-acids. Capillary, 75 cm (60 cm to detector)  $\times$  75  $\mu$ m I.D. uncoated fused silica; 0.025 mol/l borate buffer (pH 9) + 0.01 mol/l  $\beta$ -cyclodextrin; voltage, +25 kV; injection at 25 mbar for 0.1 min; capillary temperature, 30°C; detection, UV at 230 nm.

Table 3  
Statistical evaluation of the calibration graphs for the CZE determination of the aromatic sulphonic acids (peak area, arbitrary units, versus concentration, mg/l)

Compound	<i>B</i>	<i>A</i>	$r^2$	$S_{y,x}$	<i>N</i>
Chromotropic acid	0.080 ± 0.002	-0.02 ± 0.02	0.9979	0.0168	5
H-acid	0.068 ± 0.001	-1.2 ± 0.2	0.9989	0.2185	5
W-acid	0.144 ± 0.006	-0.03 ± 0.03	0.9941	0.0344	5
Koch acid	0.142 ± 0.002	-0.00 ± 0.02	0.9994	0.0233	5
Amino-F-acid	0.0807 ± 0.0005	-0.01 ± 0.02	0.9998	0.0453	8
Cleve-1,6-acid	0.0716 ± 0.0007	-0.01 ± 0.03	0.9994	0.0694	8
Cleve-1,7-acid	0.0746 ± 0.0007	-0.01 ± 0.03	0.9993	0.0628	8
Laurent acid	0.0428 ± 0.0002	0.02 ± 0.01	0.9998	0.0249	8
Peri acid	0.0432 ± 0.0005	0.01 ± 0.01	0.9995	0.0180	7

*B* is the slope of the regression lines fitted to the calibration data set ± standard deviation; *A* is the intercept of the regression lines fitted to the calibration data set ± standard deviation;  $r^2$  is the correlation coefficient of the calibration graph;  $S_{y,x}$  is the standard deviation from the calibration graph; and *N* is the number of the experimental data points.

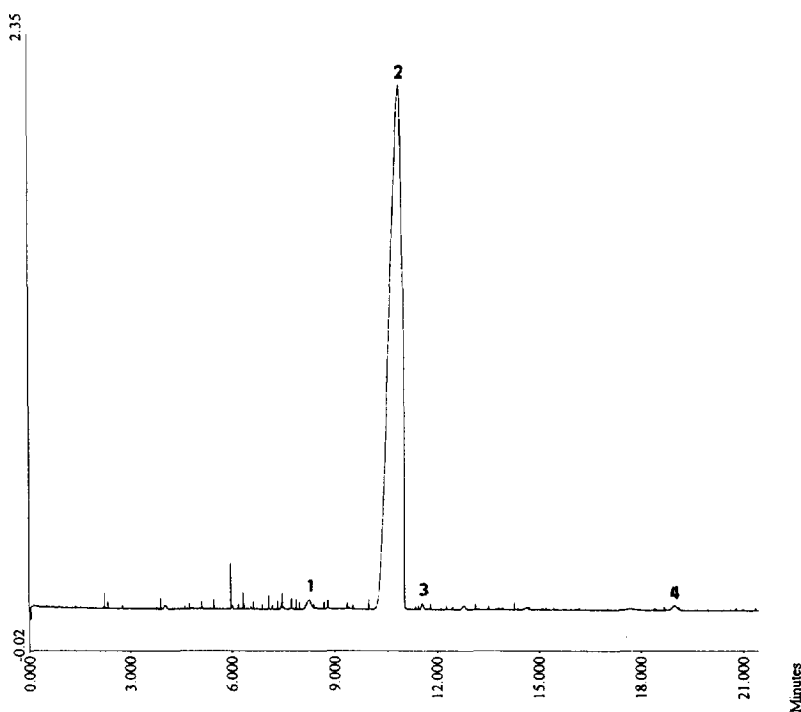


Fig. 8. CZE separation of a sample of technical H-acid. Conditions and peak numbers as in Fig. 6.

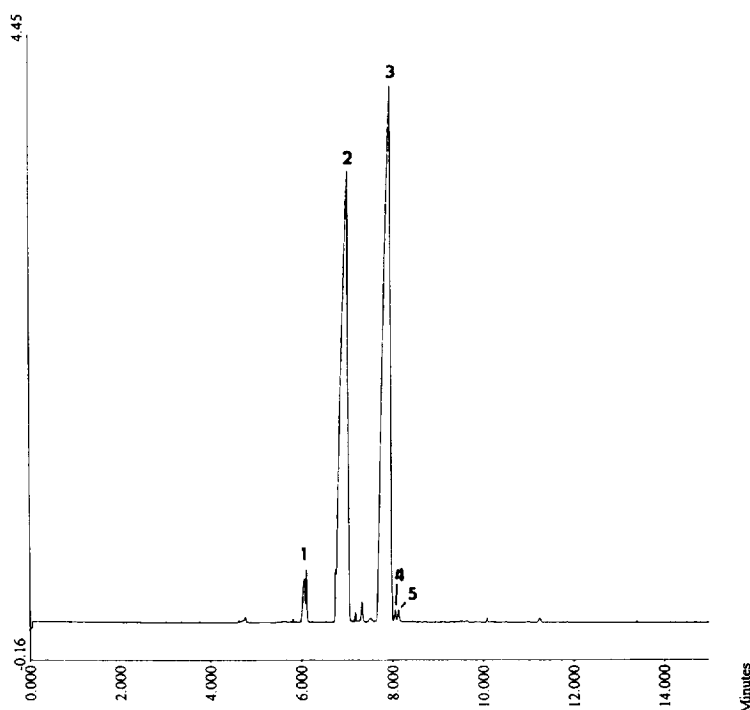


Fig. 9. CZE separation of a technological sample of Cleve acids. Conditions and peak numbers as in Fig. 5 except for injection at 25 mbar for 0.5 min.

in technical samples of Cleve acids and of H-acid.

The addition of  $\beta$ -cyclodextrin to the working electrolyte did not improve significantly the separation of the isomers of DNSA and DNBA.

#### 4. Conclusions

Reversed-phase HPLC with mobile phases containing a strong inorganic electrolyte or an alkylammonium ion-pairing reagent and CZE with working electrolytes containing  $\beta$ -cyclodextrin are complementary to each other with respect to the separation of aromatic sulphonic acids. Isomeric naphthalenesulphonic acids and a mixture of the *E* and *Z* isomers of DNSA and DNBA are best separated by HPLC with aqueous-methanolic mobile phases containing sodium sulphate. A high content of the bonded hydrocarbon moiety is essential for successful separation. Reversed-phase ion-pair chromatog-

raphy with mobile phases containing tetra-alkylammonium hydrogensulphate can be used for the analysis of some technological samples of aminonaphthalenesulphonic acids such as technical Cleve acids, but this technique may fail with other samples.

CZE with borate or phosphate buffers can separate naphthalenesulphonic acids with different numbers of sulphonic groups and some substituted aminosulphonic acids, but the migration times are long. The separation of isomers is generally inferior to the separations using reversed-phase HPLC. Addition of  $\beta$ -cyclodextrin to the working electrolyte improves the separation of isomeric naphthalenesulphonic acids, which is, however, still not as good as in reversed-phase HPLC. In phosphate, borate or mixed phosphate-borate buffers containing 0.01 mol/l  $\beta$ -cyclodextrin it is possible to separate main components and impurities in technical samples of Cleve acids (Cleve-1,6-, Cleve-1,7-, amino-F-, Laurent and peri acids), I-acids (I-,  $\gamma$ -

amino-I-, di-I-, amino-F- and dioxy-I-acids), H-acid (H-, W-, Koch and chromotropic acids) and dichlorosulphonylmethylpyrazolones (DCSPMP, DCSPH, DCSPA).

Both HPLC and CZE techniques are suitable for quantitative analysis of the aromatic sulphonic acids. The limits of detection are lower in HPLC, but the sensitivity of CZE is sufficient to detect and determine even minor impurities in technological samples. The methods developed were applied to the control of the purity of technical products.

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