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Separation of phenylamine- and naphthylaminesulphonic acids by reversed-phase high-performance liquid chromatography

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ABSTRACT

Mixtures of singly and multiply substituted phenylamine- and naphthylaminesulphonic acids, important intermediates in the synthesis of dyestuffs, were separated by reversed-phase liquid chromatography with UV detection. Linear regression analysis according to the retention equation $\ln k' = \ln k'_w + cC_b$ [where k'_w is the extrapolated capacity factor at $C_b = 0$ and c is mainly determined by molecular interaction between the solute and the eluent; they are constants for a given solute at a given column system; C_b is the concentration of organic modifier (v/v)] with and without experimental data at $C_b = 0$ being taken into account was carried out. The regression coefficient in the former situation is much lower than that in the latter. A linear relationship between $\ln k'_w$ (without taking the data at $C_b = 0$ into account) and the $\ln k'_w$ measured at $C_b = 0$ was obtained. The effect of the eluent acidity on k' is complex, indicating that the negatively charged, neutral and positively charged solutes simultaneously contribute to the retention. The retention value increases with increasing concentration of sodium chloride, but the linear regression analysis according to Horváth's equation $\ln k' = A + Bm$ (where A and B are constants related to the physico-chemical behaviour of a given column system and m is the concentration of the inorganic salt) is not good, which may be caused by masking of the ionized silanol group and changing the eluent surface tension simultaneously.

INTRODUCTION

High-performance liquid chromatography (HPLC) is the method of choice for the routine determination of ionic aromatic sulphonates [1–5]. Separations on ion exchangers are unsatisfactory because of the poor recovery of the analytes [6] and the use of a silica column with an aqueous phase leads to irreproducible retention times [1] and unstable baselines [7]. Reversed-phase columns, in contrast, give reproducible separations and stable baselines. Several separations in the ion-pair mode have been reported [8–10], but the use of an ion-pair reagent seems to shorten the column lifetime. Better, predictable and more economical separations are observed using reversed-phase columns with inorganic electrolytes as the mobile phase. We have extended this idea to determine individual phenylamine- and naphthylaminesulphonic acids and -sulphonates in complex mixtures by isocratic elution from reversed-phase columns. The effects of the concentration of organic modifier, inorganic salt and acidity on the retentions of the phenylamine- and naphthylaminesulphonic acids were investigated.

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EXPERIMENTAL

Materials

The compounds analysed (Table I) were obtained from the Dyestuff Laboratory, Chemical Engineering Department, Dalian University of Science and Technology. Standard solutions were prepared in 10 mmol/l sodium phosphate buffer (pH 6.8). Doubly distilled water was used throughout. The reagents used were of analytical-reagent grade.

Apparatus and assay conditions

HPLC was done at room temperature using stainless-steel columns (200 × 4.0 mm I.D.) that contained a reversed-phase packing material of 5-μm mean particle diameter (Polygosil 5 C_{18}) obtained from Macherey-Nagel (Düren, F.R.G.). The mobile phase was delivered by a BT-3020 pump (Biotronik, F.R.G.). The eluates were detected with a UV detector (Knauer) set at 254 nm. Samples were loaded with a Model 7125 syringe-loading sample injector (Rheodyne, U.S.A.). The flow-rate of the eluent was 1.0 ml/min. The eluent pH was measured with an SA-720 pH meter (Orion, U.S.A.). All the experimental data were processed with an NEC-APCIV personal computer.

TABLE I

PHENYLAMINE- AND NAPHTHYLAMINESULPHONIC ACIDS ANALYSED AND THEIR CAPACITY FACTORS WITH AQUEOUS BUFFER CONTAINING 10 mmol/l NaH_2PO_4 (pH 6.8) AS THE ELUENT

No.	Solute	Capacity factor (k')
1	Phenylamine-2-sulphonic acid	2.31
2	Phenylamine-3-sulphonic acid	1.03
3	Phenylamine-4-sulphonic acid	0.484
4	Phenylamine-2,5-disulphonic acid	0.078
5	5-Methylphenylamine-2-sulphonic acid	8.29
6	4-Methylphenylamine-3-sulphonic acid	2.87
7	4-Methoxyphenylamine-3-sulphonic acid	1.43
8	6-Chlorophenylamine-3-sulphonic acid	8.64
9	4-Chlorophenylamine-3-sulphonic acid	1.87
10	1,3-Diaminophenyl-4-sulphonic acid	0.510
11	1,3-Diaminophenyl-4,6-disulphonic acid	0.093
12	1,3-Diamino-2,4,6-trimethylphenyl-5-sulphonic acid	1.72
13	1,4-Diaminophenyl-2-sulphonic acid	0.612
14	2,2'-Diaminodiphenyl-4,4'-disulphonic acid	1.08
15	Naphthylamine-4-sulphonic acid	14.44
16	Naphthylamine-5-sulphonic acid	4.37
17	Naphthylamine-7-sulphonic acid	18.56
18	Naphthylamine-8-sulphonic acid	29.85
19	2-Aminonaphthalene-1-sulphonic acid	15.66
20	2-Aminonaphthalene-5-sulphonic acid	10.73
21	2-Aminonaphthalene-4,8-disulphonic acid	0.179
22	2-Aminonaphthalene-3,6-disulphonic acid	1.07
23	2-Aminonaphthalene-4,6,8-trisulphonic acid	0.027
24	2-Aminonaphthalene-3,6,8-trisulphonic acid	0.032

RESULTS AND DISCUSSION

It was known [2,7] that ionic aryl sulphonates can be separated by reversed-phase HPLC. We confirmed this and showed that singly and multiply substituted phenylamine- and naphthylaminesulphonic acids can be separated from the intermediates of synthetic dyestuffs and determined (Fig. 1, Table I). Figs. 1 and 2 show the separation of singly and multiply substituted phenylamine- and naphthylaminesulphonic acids.

Effect of molecular structure on the capacity factor, k'

From the retention data shown in Table I, the rules for the effect of molecular structure on the retention values can be deduced as follows:

(a) It is easy to separate the *o*-, *m*- and *p*-isomers of phenylamine- and naphthylaminesulphonic acids by reversed-phase (RP) HPLC. The retention data for the singly amino- and sulphonic acid-substituted phenylamine- and naphthylaminesulphonic acid isomers (Nos. 1–3 and 15–20 in Table I) support this idea.

(b) The retention order of the *o*-, *m*- and *p*-phenylaminesulphonic acid isomers is *o*- > *m*- > *p*-. The fact that the *o*-isomer has the largest retention may be due to the intramolecular hydrogen-bonding interaction, which will decrease the interaction between the solute and the mobile phase. The retention of the *m*-isomer is larger than that of the *p*-isomer, possibly owing to electrostatic effects of the substituted functional

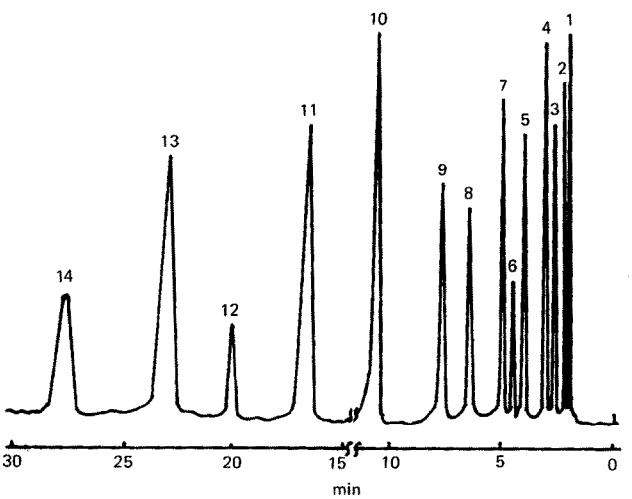


Fig. 1. Typical chromatogram of a mixture of phenylamine- and naphthylaminesulphonic acids. Mobile phase containing 10 mmol/l sodium phosphate buffer (pH 6.8). The flow-rate of the eluent was changed from 1.0 to 1.8 ml/min after sample injection at 13 min. For other conditions, see Experimental. Peaks: 1 = 2-aminonaphthalene-4,6,8-trisulphonic acid; 2 = 2-aminonaphthalene-4,8-disulphonic acid; 3 = 1,3-diaminophenyl-4-sulphonic acid; 4 = 1,4-diaminophenyl-2-sulphonic acid; 5 = 2-aminonaphthalene-3,6-disulphonic acid; 6 = 4-methoxyphenylamine-3-sulphonic acid; 7 = 1,3-diamino-2,4,6-trimethylphenyl-5-sulphonic acid; 8 = phenylamine-2-sulphonic acid; 9 = 4-methylphenylamine-3-sulphonic acid; 10 = naphthylamine-5-sulphonic acid; 11 = 6-chlorophenylamine-3-sulphonic acid; 12 = 2-aminonaphthalene-6-sulphonic acid; 13 = naphthylamine-4-sulphonic acid; 14 = naphthylamine-7-sulphonic acid.

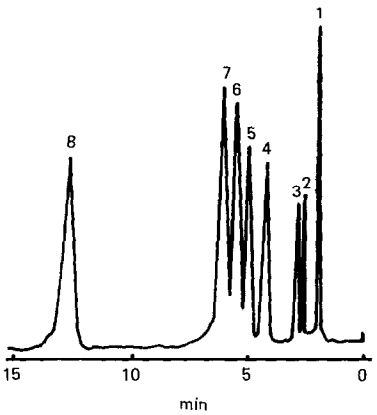


Fig. 2. Chromatogram of a mixture of eight phenylamine- and naphthylaminesulphonic acids. Mobile phase: methanol–phosphate buffer (15:85) containing 10 mmol/l NaH_2PO_4 (pH 6.8). Other experimental conditions as in Fig. 1. Peaks: 1 = 2-aminonaphthalene-3,6,8-trisulphonic acid; 2 = phenylamine-3-sulphonic acid; 3 = 1,3-diamino-2,4,6-trimethylphenyl-5-sulphonic acid; 4 = 4-methylphenylamine-3-sulphonic acid; 5 = 2-aminonaphthalene-6-sulphonic acid; 6 = 4-nitrophenylamine-2-sulphonic acid; 7 = 2-aminonaphthalene-1-sulphonic acid; 8 = naphthylamine-8-sulphonic acid.

groups, the explanation of which is as follows: the sulphonic acid group is an electronwithdrawing group in a conjugate molecule, which will attract the lone pair of electrons of the amino group in the *m*-isomer more strongly than that in the *p*-isomer. Therefore, the hydrogen-bonding interaction between the *p*-isomer and the mobile phase is stronger than that between the *m*-isomer and the mobile phase, which makes the retention of the *m*-isomer larger than that of the *p*-isomer. On the other hand, the retention of naphthylamine-8-sulphonic acid is the largest of all of the isomers of amino- and sulphonic acid-substituted naphthalenes, which means that the intramolecular hydrogen-bonding interaction between the 1- and 8-substituted groups is the strongest of all such hydrogen-bonding interactions of the tested solutes.

(c) In reversed-phase chromatography the retention of a solute increases with increasing number of non-polar groups such as CH_3, $=CH_2$ and C_6H_5, and decreases with increasing number of polar groups such as NH_2, OH and SO_3H. From the retention values in Table I, it can be seen that the negative effect of the SO_3H group on the retention value is larger than that of the NH_2 group, and the positive effects of a phenyl ring and a naphthyl ring on the retention values are approximately equal to the negative effects of two and three SO_3H groups, respectively, phenylamine-sulphonic acids substituted with NH_2 and SO_3H groups with no intramolecular hydrogen-bonding interactions still show a slight retention and naphthylaminesulphonic acids substituted with three SO_3H and one NH_2 groups show no retention under the experimental conditions used.

Effect of the organic modifier concentration on k'

The effect of variation of varying the organic modifier concentration with methanol-to-buffer volume ratios (v/v) in the range 0–0.25 on *k'* are shown in Table II.

TABLE II

CAPACITY FACTORS OF EIGHT PHENYLAMINE- AND NAPHTHYLAMINESULPHONIC ACIDS MEASURED AT DIFFERENT CONCENTRATIONS OF METHANOL IN THE MOBILE PHASE CONTAINING 10 mmol/l NaH_2PO_4 (pH 6.8)

Solute	Methanol-to-buffer ratio (v/v)					
	0	0.05	0.1	0.15	0.2	0.25
2-Aminonaphthalene-3,6,8-trisulphonic acid	0.03	0.02	0	0	0	0
Phenylamine-3-sulphonic acid	1.03	0.59	0.44	0.33	0.26	0.21
4-Methylphenylamine-3-sulphonic acid	2.97	1.05	0.77	0.57	0.42	0.32
4-Methoxyphenylamine-2-sulphonic acid	6.62	2.93	1.86	1.25	0.93	0.74
4-Nitrophenylamine-2-sulphonic acid	9.51	4.54	2.95	1.97	1.60	1.11
2-Aminonaphthalene-6-sulphonic acid	12.02	4.84	2.72	1.66	1.28	0.86
2-Aminonaphthalene-1-sulphonic acid	15.95	6.32	3.63	2.35	1.74	1.13
Naphthylamine-8-sulphonic acid	30.00	14.03	8.72	6.01	4.71	3.64

The effect of the organic modifier concentration on k' in RP-HPLC can be described as [11–13]:

$$\ln k' = \ln k'_w + cC_b \quad (1)$$

where k'_w is the capacity factor extrapolated at $C_b = 0$, c is a constant mainly determined by the molecular interaction between the solute and the eluent and C_b is the concentration of the organic modifier. The results of linear regression analysis of the experimental data shown in Table II according to eqn. 1, either not taking or taking into account the k' value at $C_b=0$, are given in Table III. It can be seen that the

TABLE III

COEFFICIENTS OF LINEAR REGRESSION ANALYSIS OF THE EXPERIMENTAL DATA IN TABLE II

Solute	Not taking account of data at $C_b=0$			Taking account of data at $C_b=0$		
	Ln k'_w	c	r	Ln k'_w	c	r
Phenylamine-3-sulphonic acid	−0.2954	−5.184	0.9976	−0.1252	−6.112	0.9840
4-Methylphenylamine-3-sulphonic acid	0.3384	−5.966	0.9997	0.7317	−8.106	0.9564
4-Methoxyphenylamine-2-sulphonic acid	1.343	−6.891	0.9909	1.629	−8.455	0.9747
4-Nitrophenylamine-2-sulphonic acid	1.802	−6.934	0.9948	2.036	−8.156	0.9811
2-Aminonaphthalene-6-sulphonic acid	1.899	−8.418	0.9906	2.207	−10.10	0.9785
2-Aminonaphthalene-1-sulphonic acid	2.187	−8.357	0.9951	2.492	−10.02	0.9803
Naphthylamine-8-sulphonic acid	2.882	−6.626	0.9891	3.154	−8.109	0.9745

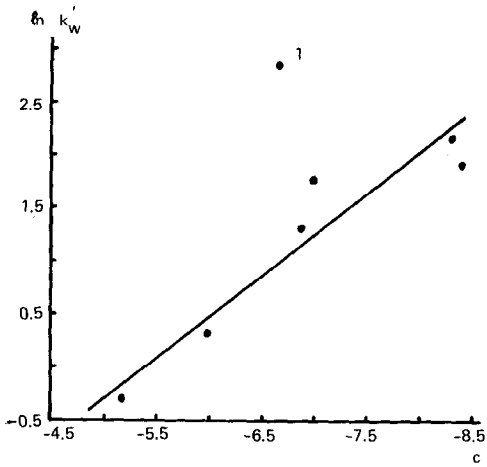


Fig. 3. Linear regression of ln k'_w *vs.* c for the six solutes excluding naphthylamine-8-sulphonic acid shown in Table III. Ln $k'_w = -3.766 - 0.7155c$, $r = 0.9343$. Point 1 represents naphthylamine-8-sulphonic acid.

regression coefficients in the former instance are much higher than those in the latter, which means that the change of k' value in the C_b range 0–0.05 is different from that in the range 0.05–0.25. There are many reports [12,14,15] that there is a linear relationship between ln k'_w and c for the same class or related compounds. Fig. 3 shows the relationship between ln k'_w and c for phenylamine- and naphthylamine sulphonic acids, and it can be seen that it is approximately linear except for naphthylamine-8-sulphonic acid, which means that the interaction behaviour of this acid is different from that of the other compounds in Table III. This may be due to the difference in the hydrogen-bonding interactions between the naphthylamine-8-sulphonic acid and the

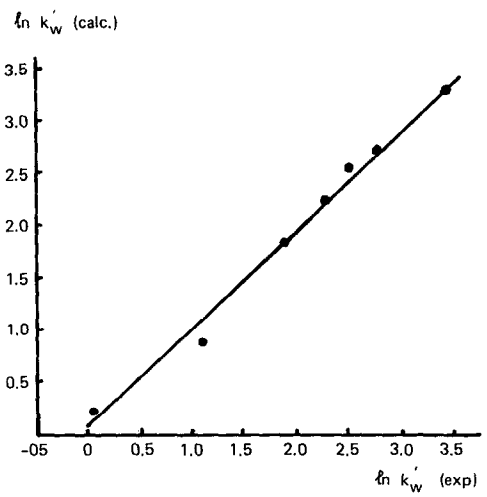


Fig. 4. Linear relationship between ln k'_w experimentally observed at $C_b = 0$ and the value extrapolated from the retention equation ln $k' = \ln k'_w + cC_b$.

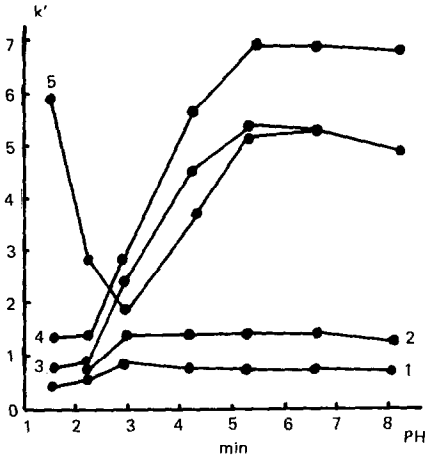
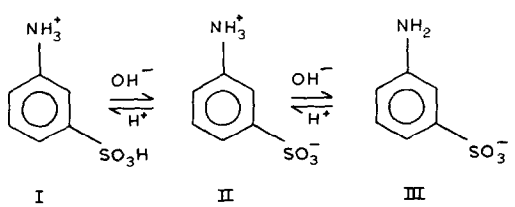


Fig. 5. Graphs showing the effect of the eluent pH on the capacity factors for five phenylamine and naphthylamine sulphonic acids. Mobile phase: methanol–phosphate buffer (0.075:0.925) containing 0.4 mol/l NaCl and 10 mol/l NaH_2PO_4. For other conditions, see Experimental. Solutes: 1 = phenylamine-3-sulphonic acid; 2 = 4-chlorophenylamine-3-sulphonic acid; 3 = 2-aminonaphthalene-6-sulphonic acid; 4 = 2-aminonaphthalene-5-sulphonic acid; 5 = 4-nitrophenylamine-2-sulphonic acid.

other compounds in the experimental column system. Some workers [14–16] used the extrapolated capacity factor ln k'_w (or log k'_w) to predict the *n*-octanol–water partition coefficient (log P_{oct}). We applied a linear regression between the extrapolated capacity factors ln k'_w on the left in Table III and those calculated from k'_w at $C_b = 0$ in Table II; the results are shown in Fig. 4 and indicate that the interaction behaviour in the real buffer system is parallel to that of the extrapolated system for the same class or related compounds.

Effect of pH of the eluent on k'

The effects of varying the eluent pH on the capacity factors of five phenylamine- and naphthylaminesulphonic acids are shown in Fig. 5. Solutions covering the pH range 1.4–8.2 were adjusted by adding 6 mol/l HCl to an eluent containing 10 mmol/l KH_2PO_4 and 0.4 mol/l NaCl with $C_b = 0.075$, where the high concentration of NaCl is intended to minimize the effect on the capacity factor of ionic strength changes caused by variations in pH. Over this pH range, the solutes containing NH_2 and SO_3H groups can exist in three different ionization states:



I II III

The pK_a value of the NH_2 group is *ca.* 4.5 and that of the SO_3H group is 1–2.5. In the pH range 5–8.2 there is no serious change in the capacity factors of any of the solutes,

TABLE IV

CAPACITY FACTORS OF 7 PHENYLAMINE- AND NAPHTHYLAMINESULPHONIC ACIDS MEASURED AT DIFFERENT CONCENTRATIONS OF INORGANIC SALT IN THE MOBILE PHASE CONTAINING 10 mmol/l NaH_2PO_4 (pH 6.8) WITH $C_b = 0.08$

Solute	NaCl (mmol/l)					
	0	0.91	50	10.9	350	609
2-Aminonaphthalene-3,6,8-trisulphonic acid	0.03	0.07	0.12	0.14	0.17	0.19
Phenylamine-3-sulphonic acid	0.55	0.57	0.64	0.68	0.74	0.78
4-Methylphenylamine-3-sulphonic acid	0.91	0.99	1.09	1.17	1.32	1.40
4-Methoxyphenylamine-2-sulphonic acid	2.29	2.49	2.78	2.95	3.36	3.63
4-Nitrophenylamine-2-sulphonic acid	3.58	3.88	4.26	4.55	4.94	5.22
2-Aminonaphthalene-6-sulphonic acid	3.36	3.58	3.98	4.27	4.66	4.97
2-Aminonaphthalene-1-sulphonic acid	4.61	5.00	5.56	5.93	6.67	7.27

because they exist in form III. However, with decrease in the pH of the eluent, the NH_2 group becomes partly ionized to NH_3^+. In the pH range 3–5, solutes 3, 4 and 5 in Fig. 5 exist in forms II and III and the capacity factor decreases, but there is no serious change in the capacity factors for solutes 1 and 2, which means these two solutes are still in form III. With a further decrease in pH value to 1.4–3, the NH_2 group in solutes 3, 4 and 5 is fully ionized to NH_3^+, and the SO_3^- of solute 5 is partly non-ionized as SO_3H, which means that solutes 3 and 4 exist in form II and solute 5 in the forms I and II. However, solutes 1 and 2 may exist in forms II and III.

Effect of inorganic salt concentration on k'

The variation of k' with varying concentration of the inorganic salt is shown in Table IV. The capacity factors of the phenylamine- and naphthylaminesulphonic acids increase with increasing concentration of NaCl in the eluent. According to Horváth *et al.* [17], the effect of the inorganic salt concentration on k' in RP-HPLC can be expressed as

$$\ln k' = A + Bm \tag{2}$$

TABLE V

COEFFICIENTS OF LINEAR REGRESSION ANALYSIS OF THE EXPERIMENTAL DATA IN TABLE IV

Solute	*A*	*B*	*r*
2-Aminonaphthalene-3,5,7-sulphonic acid	−2.599	1.865	0.7117
Phenylamine-3-sulphonic acid	−0.5220	0.5071	0.8929
4-Methylphenylamine-3-sulphonic acid	0.0116	0.6111	0.9062
4-Methoxyphenylamine-2-sulphonic acid	0.9343	0.6569	0.9142
4-Nitrophenylamine-2-sulphonic acid	1.377	0.5193	0.8814
2-Aminonaphthalene-6-sulphonic acid	1.308	0.5260	0.8773
2-Aminonaphthalene-1-sulphonic acid	1.643	0.6468	0.9167

where A and B are constants related to the physico-chemical behaviour of a given column system and m is the concentration of the inorganic salt. Table V gives the coefficients of the linear regression analysis of the experimental data in Table IV. It can be seen that the regression coefficient is about 0.9, which means that the linear relationship represented by eqn. 2 is not very good. We consider that the k' values of the solutes are affected not only by the surface tension of the eluent with varying salt concentration, but also by the interaction between the NH_2 and SO_3^- groups and ionized silanol groups [18]. With increasing NaCl concentration, the ionized silanol group is partly masked, which will decrease the replusive interaction between the ionized silanol group and the SO_3^- group of the solute and increase its capacity factor. Therefore, the linear relationship in eqn. 2 is not good enough to describe the effects of the NaCl concentration on k'.

CONCLUSION

The proposed method enabled us to determine the phenylamine- and naphthylaminesulphonic acids in the synthesis of dyestuffs on a reversed-phase column. The retention values are affected by the concentration of organic modifier and inorganic salt and the pH of the eluent. A linear relationship between the experimentally measured $\ln k'_w$ values and those extrapolated from the retention equation $\ln k' = \ln k'_w + cC_b$ was observed.

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