

# Retention behaviour of aromatic sulphonic acids in reversed-phase ion-pair liquid chromatography with methanol and acetonitrile as organic modifiers

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## ABSTRACT

The capacity factors of phenylamine- and naphthylaminesulphonic acids in reversed-phase ion-pair liquid chromatography were measured. The equations  $\log k' = \log k_w - S\varphi$  and  $\log k' = \log k_w + A\varphi^2 - S\varphi$  to describe the effects of methanol and acetonitrile concentration were compared. It has been observed that the effect of organic modifier concentration up to  $\varphi = 0$  on retention follows the empirical equation  $\log k' = \log k_w - S\varphi$ . It was found that the effect of acetonitrile on retention is stronger than that of methanol, which is the same as the order of their elution strengths observed in RP-HPLC.

## INTRODUCTION

Reversed-phase ion-pair liquid chromatography (RP-IPC) is widely used in separations of organic and inorganic ions. Retention can be regulated by the properties and concentrations of the organic modifier and counter ion and by using a competing ion with the same charge as the analyte. Many models of RP-IPC have been published [1–7] and excellent reviews have appeared [1,8]. In recent years, the electrostatic model with the use of the Gouy–Chapman theory has been applied to IPC [9–14]. The effects of chromatographic variables such as organic modifier concentration [13], ion-pair reagent [15], inorganic salt concentration [16], column temperature [17] and solute properties such as hydrophobicity and charge [18,19] on retention have been investigated. It has been observed that both hydrophobicity and solute charge play a very important role in retention in RP-IPC and in most instances the retention

order in RP-IPC for organic ions with the same charge is the same as in RP-HPLC [20]. In this work, the retention behaviour of sulphonic acids in RP-IPC with tetrabutylammonium iodide as ion-pair reagent and methanol and acetonitrile as organic modifiers was studied.

## EXPERIMENTAL

### Materials

The phenylamine- and naphthylaminesulphonic acids (Table I) were obtained from the Dyestuff Laboratory, Chemical Engineering Department, Dalian University of Sciences and Technology. Standard solutions were prepared in water. Doubly distilled water was used throughout. Methanol, tetrabutylammonium iodide (TBAI),  $\text{NaH}_2\text{PO}_4$ , NaOH, KCl and HCl were of analytical-reagent grade.

### Apparatus

RP-IPC experiments were carried out on a stainless-steel column ( $150 \times 4.6$  mm I.D.) packed with Spherisorb- $\text{C}_{18}$  (10  $\mu\text{m}$ ) (Phase

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Separations, Deeside, UK) at room temperature (26°C). The column was packed at the National Chromatographic R & A Centre, Dalian, China. The mobile phases contained methanol, acetonitrile and water in different proportions with constant concentrations of TBAI ion-pair reagent (4 mmol/l),  $\text{NaH}_2\text{PO}_4$  (10 mmol/l) and KCl (10 mmol/l) and with a pH of 7.00. Mobile phase was delivered with a Waters (Milford, MA, USA) Model 510 pump. Eluates were detected at 254 nm. Samples were loaded with a U6K syringe-loading sample injector. The pH value of the organic modifier solution was measured with an SA-720 pH meter (Orion Research, Chicago, IL, USA). As discussed by Karger *et al.* [21], with up to 25% of organic modifier in the aqueous eluent, the systematic error in pH measured pH in a mixed solvent and standardizing with aqueous buffer is much less than 0.1 pH unit. The flow-rate was 1.0 ml/min. All experimental data was processed using a personal computer.

## RESULTS AND DISCUSSION

### Relationship between retention and organic modifier concentration

The retention times of each sulphonic acid were measured twice with different methanol

and acetonitrile concentrations from 0.22 to 0 volume fraction and the difference between the two retention times in each instance was less than 3%. Pre-equilibration of the column for 30 min was applied when changing the mobile phase composition. The capacity factors of test solutes calculated from retention times are given in Tables I and II. In reversed-phase high-performance liquid chromatography (RP-HPLC), according to the solubility parameter concept [22], the relationship between solute retention and organic modifier concentration can be described by

$$\log k' = \log k_w + A\varphi^2 - S\varphi \quad (1)$$

where  $\log k_w$  is the capacity factor obtained by extrapolation of retention data from binary eluents to 100% water,  $A$  and  $S$  are constants for a given solute–eluent combination and  $\varphi$  is the volume fraction of the organic modifier in the aqueous eluent. Snyder *et al.* [23] showed that over a volume fraction range of at most 0.1–0.9, eqn. 1 can be simplified as a good approximation to

$$\log k' = \log k_w - S\varphi \quad (2)$$

In RP-IPC, a retention equation identical with eqn. 2 was used to describe the effect of organic modifier concentration on retention. The results

TABLE I

CAPACITY FACTORS OF PHENYLAMINE- AND NAPHTHYLAMINESULPHONIC ACIDS AT DIFFERENT METHANOL CONCENTRATIONS

Solute	Volume fraction of methanol, $\varphi(\text{v/v})$				
	0.22	0.15	0.08	0.04	0
1,3-Diaminobenzene-4-sulphonic acid	0.130	0.420	0.712	0.987	1.52
1-Aminobenzene-4-sulphonic acid	0.146	0.419	0.699	0.936	1.56
1-Aminobenzene-3-sulphonic acid	0.394	0.885	1.81	2.80	4.31
1,3-Diaminobenzene-4,6-disulphonic acid	0.441	1.02	2.70	4.00	6.91
1-Amino-4-methylbenzene-2-sulphonic acid	0.842	1.97	4.19	6.74	10.21
1-Aminonaphthalene-5-sulphonic acid	1.26	3.02	8.10	13.08	23.07
2-Aminonaphthalene-4,7-disulphonic acid	1.72	4.81	17.72	35.17	68.95
2-Aminonaphthalene-4,8-disulphonic acid	1.64	5.37	18.26	36.48	74.11
2-Aminonaphthalene-5-sulphonic acid	3.57	9.45	29.33	50.56	90.52
2-Aminonaphthalene-3,6-disulphonic acid	2.24	8.08	31.08	87.33	133.9
2-Aminonaphthalene-4,6,8-trisulphonic acid	2.95	10.02	45.19	95.57	191.3
2-Aminonaphthalene-3,6,8-trisulphonic acid	3.70	10.25	49.15	102.0	201.6

TABLE II

CAPACITY FACTORS OF PHENYLAMINE- AND NAPHTHYLAMINESULPHONIC ACIDS AT DIFFERENT ACETONITRILE CONCENTRATIONS

Solute	Volume fraction of acetonitrile, $\phi(v/v)$				
	0.22	0.15	0.08	0.04	0
1,3-Diaminobenzene-4-sulphonic acid	0.090	0.224	0.539	0.769	1.52
1-Aminobenzene-4-sulphonic acid	0.077	0.256	0.571	0.833	1.56
1-Aminobenzene-3-sulphonic acid	0.122	0.474	1.20	1.98	4.31
1,3-Diaminobenzene-4,6-disulphonic acid	0.135	0.635	1.80	3.09	6.91
1-Amino-4-methylbenzene-2-sulphonic acid	0.224	0.737	2.40	4.39	10.21
1-Aminonaphthalene-5-sulphonic acid	0.410	1.36	4.52	9.39	23.07
2-Aminonaphthalene-4,7-disulphonic acid	0.378	1.98	8.06	18.75	68.95
2-Aminonaphthalene-4,8-disulphonic acid	0.330	1.66	7.84	19.65	74.11
2-Aminonaphthalene-5-sulphonic acid	0.660	2.76	13.19	33.33	90.52
2-Aminonaphthalene-3,6-disulphonic acid	0.474	2.43	12.98	34.36	133.9
2-Aminonaphthalene-4,6,8-trisulphonic acid	0.680	4.24	24.04	53.41	191.3
2-Aminonaphthalene-3,6,8-trisulphonic acid	0.756	4.40	25.49	60.12	201.6

TABLE III

LOG  $k_w$ ,  $S$  AND  $A$  IN EQN. 1 OBTAINED BY REGRESSION ANALYSIS OF THE EXPERIMENTAL DATA IN TABLES I AND II

Solute	Methanol–water				Acetonitrile–water			
	Log $k_w$	$S$	$A$	$r$	Log $k_w$	$S$	$A$	$r$
1,3-Diaminobenzene-4-sulphonic acid	0.148	2.496	−9.528	0.9936	0.157	5.593	0.730	0.9980
1-Aminobenzene-4-sulphonic acid	0.155	3.066	−6.030	0.9924	0.160	4.467	−5.621	0.9970
1-Aminobenzene-3-sulphonic acid	0.631	4.490	−0.902	0.9994	0.596	5.962	−3.639	0.9971
1,3-Diaminobenzene-4,6-disulphonic acid	0.836	5.365	−0.320	0.9993	0.800	6.070	−6.430	0.9974
1-Amino-4-methylbenzene-2-sulphonic acid	1.009	4.590	−1.482	0.9999	0.991	7.923	2.263	0.9994
1-Aminonaphthalene-5-sulphonic acid	1.362	5.939	0.865	0.9998	1.348	9.034	5.374	0.9997
2-Aminonaphthalene-4,7-disulphonic acid	1.851	8.027	2.883	0.9995	1.790	11.38	6.572	0.9979
2-Aminonaphthalene-4,8-disulphonic acid	1.870	7.708	0.828	1.0000	1.833	12.39	8.848	0.9989
2-Aminonaphthalene-5-sulphonic acid	1.962	6.437	−0.004	0.9996	1.946	10.93	5.782	0.9999
2-Aminonaphthalene-3,6-disulphonic acid	2.172	7.983	−1.662	0.9971	2.096	13.10	9.857	0.9993
2-Aminonaphthalene-4,6,8-trisulphonic acid	2.299	8.403	0.093	0.9993	2.243	11.11	0.987	0.9987
2-Aminonaphthalene-3,6,8-trisulphonic acid	2.333	8.878	3.391	0.9979	2.279	11.29	1.893	0.9995

of regression analysis of the experimental data shown in Tables I and II according to eqn. 1 are given in Table III. The plots of  $\log k'$  vs.  $\varphi$  according to eqn. 2 for the data in Tables I and II are given in Figs. 1 and 2; Tables IV and V give the values of  $\log k_w$  and  $S$  in eqn. 2 obtained by taking or not taking into account, respectively, the  $k'$  values at  $\varphi = 0$ .

It can be seen that the regression coefficients with eqns. 1 and 2 are higher than 0.99 for all solutes, except for 1-aminobenzene-4-sulphonic acid and 1,3-diaminobenzene-4-sulphonic acid in Table IV, the reason for which is probably the low numerical value of  $k'$ , giving a high uncertainty. The values of  $\log k_w$  and  $S$  obtained with eqn. 1 are close to those given by eqn. 2 with methanol as the organic modifier; their relative difference is less than 10% for ten of the twelve solutes. However, the relative difference between the values of  $S$  obtained with eqns. 1 and

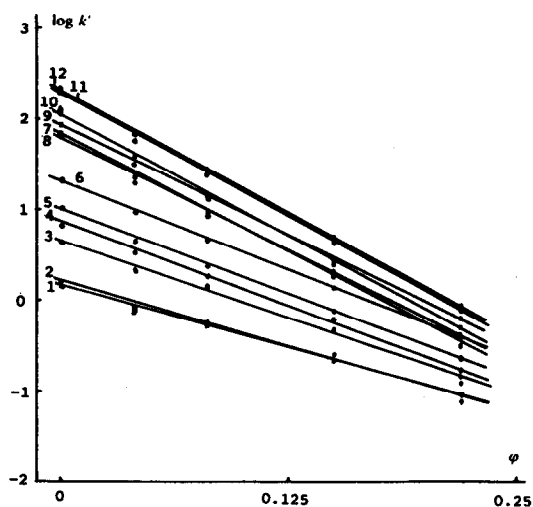


Fig. 2. Plots of  $\log k'$  vs.  $\varphi$  according to eqn. 2 with acetonitrile as organic modifier. Compound numbers as in Fig. 1.

2 is larger than 10% for eight of the twelve solutes with acetonitrile as the organic modifier. The regression coefficients with eqn. 1 are only slightly higher than those with eqn. (2), which means that both eqns. 1 and 2 describe well the effect of organic modifier concentration on retention in our experimental system. The value of  $A$  in eqn. 1 varies from 3.391 to  $-9.528$  with methanol and from  $-6.430$  to  $9.857$  with acetonitrile as the organic modifier. The reason is probably the minor statistical weight in regression analysis giving high uncertainty to the  $A$  values. The  $\varphi^2$  term in eqn. 1 makes a much smaller contribution to the retention than the  $\varphi$  term does. In general, the effect of the  $\varphi^2$  term on retention with acetonitrile as an organic modifier is stronger than that with methanol as an organic modifier. The regression coefficients with eqn. 1 considering or not considering the data at  $\varphi = 0$  are fairly close and the relative differences in the parameters  $\log k_w$  and  $S$  between two instances are less than 5% in most cases. The above results mean that eqn. 2 can accurately describe the effect of organic modifier concentration on retention up to  $\varphi = 0$  in our experimental system, but eqn. 2 is a better approximation for methanol than for acetonitrile, which is usually observed in RP-HPLC.

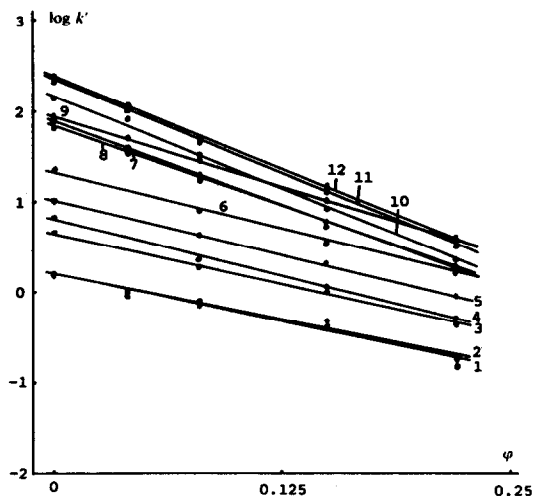


Fig. 1. Plots of  $\log k'$  vs.  $\varphi$  according to eqn. 2 with methanol as organic modifier. 1 = 1,3-Diaminobenzene-4-sulphonic acid; 2 = 1-aminobenzene-4-sulphonic acid; 3 = 1-aminobenzene-3-sulphonic acid; 4 = 1,3-diaminobenzene-4,6-disulphonic acid; 5 = 1-amino-4-methylbenzene-2-sulphonic acid; 6 = 1-aminonaphthalene-5-sulphonic acid; 7 = 2-aminonaphthalene-4,7-disulphonic acid; 8 = 2-aminonaphthalene-4,8-disulphonic acid; 9 = 2-aminonaphthalene-5-sulphonic acid; 10 = 2-aminonaphthalene-3,6-disulphonic acid; 11 = 2-aminonaphthalene-4,6,8-trisulphonic acid; 12 = 2-aminonaphthalene-3,6,8-trisulphonic acid.

TABLE IV

LOG  $k_w$  AND  $S$  IN EQN. 2 OBTAINED BY LINEAR REGRESSION ANALYSIS OF THE EXPERIMENTAL DATA IN TABLE I

Solute	Not taking into account data at $\varphi = 0$			Taking into account data at $\varphi = 0$		
	Log $k_w$	$S$	$r$	Log $k_w$	$S$	$r$
1,3-Diaminobenzene-4-sulphonic acid	0.230	4.768	0.9793	0.205	4.614	0.9855
1-Aminobenzene-4-sulphonic acid	0.188	4.390	0.9817	0.191	4.403	0.9888
1-Aminobenzene-3-sulphonic acid	0.638	4.699	0.9997	0.636	4.691	0.9998
1,3-Diaminobenzene-4,6-disulphonic acid	0.836	5.426	0.9988	0.838	5.436	0.9993
1-Amino-4-methylbenzene-2-sulphonic acid	1.027	4.980	0.9997	1.018	4.921	0.9997
1-Aminonaphthalene-5-sulphonic acid	1.350	5.707	0.9996	1.357	5.747	0.9997
2-Aminonaphthalene-4,7-disulphonic acid	1.829	7.357	0.9987	1.834	7.386	0.9992
2-Aminonaphthalene-4,8-disulphonic acid	1.860	7.493	1.0000	1.865	7.524	1.0000
2-Aminonaphthalene-5-sulphonic acid	1.968	6.474	0.9995	1.962	6.438	0.9996
2-Aminonaphthalene-3,6-disulphonic acid	2.240	8.712	0.9978	2.182	8.352	0.9971
2-Aminonaphthalene-4,6,8-trisulphonic acid	2.317	8.496	0.9991	2.299	8.382	0.9993
2-Aminonaphthalene-3,6,8-trisulphonic acid	2.321	8.179	0.9961	2.313	8.124	0.9975

#### Relationship of retention values in different mobile phases

Cross-comparison of the log  $k'$  values at the same volume fraction of methanol and acetonitrile and the ion-pair reagent concentration in the eluents revealed two effects: first, the retention order of sulphonic acids was not different between the two organic modifiers, and second, sulphonic acids were retained in the column much longer by methanol–water than by acetonitrile–water mixtures. For instance, 2-amino-naphthalene-4,6,8-trisulphonic acid eluted at 45 min with methanol–water (0.08:0.92). The same compound eluted only at 24 min with acetonitrile–water (0.08:0.92). The parameter  $S$  in eqn. 2 with acetonitrile–water as the eluent is much larger than that with methanol–water, which means that there is a much stronger effect of

acetonitrile on the retention of sulphonic acids than that of methanol in RP-IPC, which agrees with the results observed by Bartha *et al.* [14] that the effect of acetonitrile on the adsorbed amount of ion-pair reagent is stronger than that of methanol, and also agrees with the order of elution strength as an organic modifier in RP-HPLC. It is clear that the hydrophobic interaction plays an important role in the adsorption of ion-pair reagents on the stationary surface and the retention of ionic solutes in RP-IPC.

The results of linear regression of the log  $k'$  values, log  $k_w$  and  $S$  between the two organic modifiers are given in Figs. 3, 4 and 5, respectively. The regression coefficients for the correlation of log  $k'_{(\text{ACN})}$  vs. log  $k'_{(\text{MeOH})}$  and log  $k_{w(\text{ACN})}$  vs. log  $k_{w(\text{MeOH})}$  are higher than 0.99, but that of  $S_{(\text{ACN})}$  vs.  $S_{(\text{MeOH})}$  is only 0.957. The lower

TABLE V

LOG  $k_w$  AND  $S$  IN EQN. 2 OBTAINED BY LINEAR REGRESSION ANALYSIS OF THE EXPERIMENTAL DATA IN TABLE II

Solute	Not taking into account data at $\varphi = 0$			Taking into account data at $\varphi = 0$		
	Log $k_w$	$S$	$r$	Log $k_w$	$S$	$r$
1,3-Diaminobenzene-4-sulphonic acid	0.123	5.245	0.9981	0.153	5.430	0.9980
1-Aminobenzene-4-sulphonic acid	0.194	5.718	0.9920	0.193	5.717	0.9951
1-Aminobenzene-3-sulphonic acid	0.601	6.667	0.9944	0.618	6.773	0.9965
1,3-Diaminobenzene-4,6-disulphonic acid	0.838	7.495	0.9935	0.839	7.501	0.9960
1-Amino-4-methylbenzene-2-sulphonic acid	0.943	7.209	0.9998	0.977	7.418	0.9993
1-Aminonaphthalene-5-sulphonic acid	1.266	7.531	0.9999	1.315	7.836	0.9988
2-Aminonaphthalene-4,7-disulphonic acid	1.659	9.353	0.9993	1.750	9.917	0.9971
2-Aminonaphthalene-4,8-disulphonic acid	1.686	9.831	1.0000	1.780	10.41	0.9975
2-Aminonaphthalene-5-sulphonic acid	1.864	9.349	0.9998	1.914	9.659	0.9991
2-Aminonaphthalene-3,6-disulphonic acid	1.943	10.33	1.0000	2.031	10.91	0.9977
2-Aminonaphthalene-4,6,8-trisulphonic acid	2.192	10.61	0.9990	2.237	10.89	0.9989
2-Aminonaphthalene-3,6,8-trisulphonic acid	2.229	10.63	0.9996	2.267	10.87	0.9994

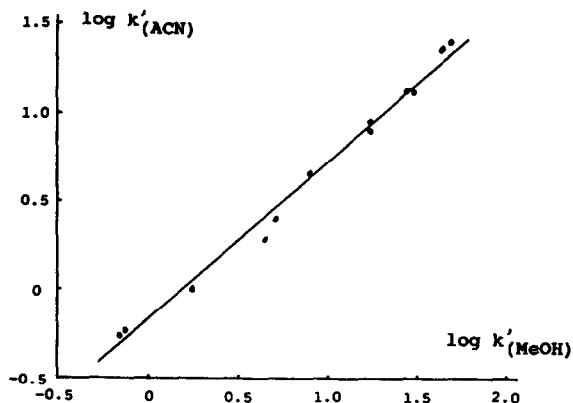


Fig. 3. Results of linear regression analysis of  $\log k'$  values between the two organic modifiers with volume fraction 0.08. For experimental conditions, see text. Regression equation:  $\log k'_{(ACN)} = -0.141 + 0.873 \log k'_{(MeOH)}$ ,  $r = 0.9972$ ,  $n = 12$ .

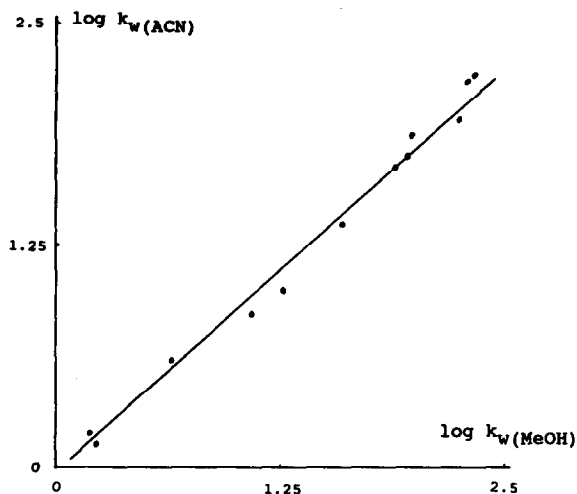


Fig. 4. Result of linear regression analysis of the  $\log k_w$  values between the two organic modifiers. For experimental conditions, see text. Regression equation:  $\log k_{w(ACN)} = -0.004 + 0.935 \log k_{w(MeOH)}$ ,  $r = 0.9964$ ,  $n = 12$ .

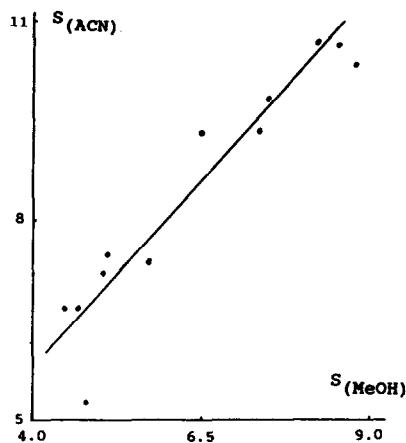


Fig. 5. Result of linear regression analysis of the  $S$  values between the two organic modifiers. For experimental conditions, see text. Regression equation:  $S_{(\text{ACN})} = 0.987 + 1.149 S_{(\text{MeOH})}$ ,  $r = 0.9568$ ,  $n = 12$ .

regression coefficient for  $S_{(\text{ACN})}$  vs.  $S_{(\text{MeOH})}$  may be caused from the facts that there is some difference in selectivity between methanol and acetonitrile mixtures and the low numerical value for some solutes such as 1,3-diaminobenzene-4-sulphonic acid resulted in an uncertainty of the  $S$  value. For example, the regression coefficient is 0.973 for the eleven solutes excluding 1,3-diaminobenzene-4-sulphonic acid.

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