

# The *S* index in the retention equation in reversed-phase high-performance liquid chromatography

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## ABSTRACT

The empirical retention equation  $\log k' = \log k'_w - S\phi$  in reversed-phase high-performance liquid chromatography (RP-HPLC) was investigated to evaluate the properties of the parameter *S*. The *S* index, which is defined as the slope of  $\log k'$  versus volume fraction of the organic modifier ( $\phi$ ) was systematically examined as a function of bonded phase density, column type and temperature in RP-HPLC. The *S* index for a particular solute was observed to be nearly constant even when column systems with different  $C_{18}$  packing materials are used. The dependence of  $\log k'$  on eluent composition was found to be represented by parallel lines for a given solute for a variety of different stationary phases. The *S* index remains constant for a given solute despite the prolonged use of  $C_{18}$  column. The results showed that the *S* index is determined mainly by the interaction between the solute and the mobile phase. It was observed to decrease with the increasing column temperature for non-ionic solutes. Other factors influencing the measurement of the *S* index are discussed.

## INTRODUCTION

The mechanism governing solute retention in reversed-phase high-performance liquid chromatography (RP-HPLC) is of considerable research interest [1–16]. Most of the proposed models have constructed a retention scheme and the corresponding equilibrium constants. By combining these constants with empirical relationships, equations are obtained for the capacity factors as a function of different variables, of which eluent composition is one of the most important factors in RP-HPLC. At present, the best and most rigorous description of RP-HPLC retention is the solvophobic model developed by Horváth and co-workers [1,2], which has explained many fundamental retention behaviours observed in RP-HPLC.

The stoichiometric displacement model (SDM), which was developed by Geng and Regnier [3,4], is

also important in the development of reversed-phase retention theory. They developed a double logarithmic plot to represent the dependence of retention on solvent composition and this has been widely applied in both RP-HPLC and hydrophobic interaction chromatography (HIC). According to the solubility parameter concept [5,6], a quadratic dependence of the retention on mobile phase composition has been derived and been predicted theoretically [14]. This general equation was found to describe precisely the retention over a wide range of concentrations of the mobile phase.

As the rigorous theoretical study of retention mechanisms is very complicated [12–16], it is more practical to approximate the retention behaviour of a solute by using empirical relationships. In RP-HPLC, the linear approximation of the relationship between the logarithm of capacity factors and the eluent composition has commonly been used and has been found to be fairly reliable in practical RP-HPLC applications [10] (see eqn. 2). However, it must be pointed that deviations from linearity have been observed and not all solvent systems are

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equally suitable [5,6]. This empirical retention equation has been shown to be strictly valid for methanol–water mobile phase.

The parameter  $S$  in the empirical retention equation plays an important role in computer simulations in RP-HPLC [11]. It has been suggested that  $S$  is a characteristic constant of an organic solvent depending only on the strength of the organic modifier used. It has been found that  $S$  is not invariant with solutes. In several studies,  $S$  tended to increase with increasing solute retention and to be a function of the functional groups in the solute [11].

This paper systematically describes the  $S$  index as a function of bonded phase density, column type and temperature based on the empirical retention equation  $\log k' = \log k'_w - S\phi$  in RP-HPLC.

#### RETENTION EQUATION TO DESCRIBE THE EFFECT OF ORGANIC MODIFIER CONCENTRATION ON CAPACITY FACTORS IN RP-HPLC

A linear approximation of the retention equation to describe the effect of organic modifier concentration on the logarithm of the capacity factor ( $k'$ ) in RP-HPLC has been widely accepted in practical RP-HPLC and no significant errors in retention prediction have been found. It is expressed as

$$\log k' = \log k'_w - S\phi \quad (1)$$

where  $\log k'_w$  and  $S$  are constants for a given column system. The  $S$  index is defined as the slope of  $\log k'$  versus volume fraction of organic modifier ( $\phi$ ). A thermodynamic explanation of  $S$  can be attempted if the free-energy of the interaction between the solute and solvent molecules is taken as a linear function of eluent composition [1,2]. We have used a thermodynamic method in combination with an empirical relationship to derive the retention equation  $\log k' = \log k'_w - S\phi$ ; the parameters  $\log k'_w$  and  $S$  can be expressed as

$$\log k'_w = \log \beta + (\Delta G_{A,C}^0 - \Delta G_{A,L}^0)/RT \quad (2)$$

$$S = (\Delta G_{A,C}^0 - \Delta G_{A,B}^0)/RT \quad (3)$$

where  $R$  is the gas constant,  $T$  is the absolute temperature,  $\beta$  is phase ratio, the subscripts A, B, C and L refer to solute, strong solvent, weak solvent and hydrocarbonaceous ligands, respectively,  $\Delta G_{A,B}^0$  and  $\Delta G_{A,C}^0$  are the non-electrostatic free-energy

changes for solute–strong solvent and solute–weak solvent, respectively, and  $\Delta G_{A,L}^0$  is the non-electrostatic free-energy change for solute–hydrocarbonaceous ligands. As can be seen,  $S$  is determined mainly by the interactions in the mobile phase. The  $S$  index characterizes the properties of the mobile phase and approaches a constant for a certain solute with different  $C_{18}$  packing materials.

The statistical mechanics theory described by Dill [14] and Lu and Lu [15] also showed that  $S$  was mainly determined by the interaction between the solute and the mobile phase.

#### EXPERIMENTAL

The liquid chromatograph was constructed in our laboratory and consisted of a YSB-2 pump (Shanghai Instrumental Plant, Shanghai, China), a Model 7010 injector (Rheodyne, Cotati, CA, USA), and a Uvidec-100-III UV detector (Jasco, Tokyo, Japan) operating at 254 nm. Reversed-phase packing materials YWG- $C_{18}$  (Tianjin Chemical Reagent Factory, Tianjin, China), ES- $C_{18}$  (ES Industries, Marlton, NJ, USA), Nucleosil- $C_{18}$ , LiChrosorb RP- $C_{18}$ , B4- $C_{18}$  and B8- $C_{18}$  with 5- $\mu$ m particle diameter were employed. B4- $C_{18}$  and B8- $C_{18}$  are laboratory-made silica-based  $C_{18}$  packing materials, prepared from monochlorodimethyloctadecylsilane. Dry silica was suspended with toluene and pyridine and within 1 h the silane agent was added, then the mixture was refluxed for 12 h. The product was washed with acetone, chloroform, acetone and methanol. Energetically homogeneously distributed  $C_{18}$  bonded phases were obtained.

We used six ODS columns (250  $\times$  4.6 mm I.D.) which were packed in our laboratory. A column (200  $\times$  4.0 mm I.D.) containing Polygosil- $C_{18}$  reversed-phase packing material with 5- $\mu$ m particle diameter was also used.

The factors influencing the reproducibility of  $k'$  on a given  $C_{18}$  column were systematically investigated.

Mobile phase mixtures were prepared from individually measured volumes of methanol and deionized water in the range 50–90%. All solutes were of analytical-reagent grade.

The column dead time,  $t_0$ , was determined using sodium nitrite as a non-retained compound. All HPLC measurements were performed at room temperature.

Other experimental results utilized in this work were taken from papers by Petrovic and Lomic [17,18], Smith and Burr [19], Braumann *et al.* [20,21], Engelhardt and co-workers [22,23], Jinno and Kuwajima [24] and Hafkenscheid and Tomlinson [25], which give exact descriptions of the analytical conditions employed.

## RESULTS AND DISCUSSION

As is well known, the variation of  $k'$  for a specific solute over a prolonged period on the  $C_{18}$  column is considerable even under closely controlled chromatographic conditions [19]. Tables I and II compare

of the reproducibilities of  $k'$  and  $S$  over a 2-year period of extensive use of a  $C_{18}$  column with methanol-water and acetonitrile-water mobile phases. The results illustrate that the prolonged use of the columns does not affect the  $S$  values for a specific solute despite a considerable variation in the retention values for each of the compounds. The reproducibility of the  $S$  index for a given solute within the same laboratory on a certain  $C_{18}$  column is generally of the order of 0.05.

It is generally observed that the retention values differ significantly when column systems with the same mobile phase concentration but with  $C_{18}$  packing materials from different sources or even

TABLE I

COMPARISON OF LONG-TERM REPRODUCIBILITIES OF  $k'$  AND  $S$  INDEX OVER A 2-YEAR PERIOD WITH METHANOL-WATER AS MOBILE PHASE

Column, Spherisorb  $C_{18}$ ; eluent, methanol-buffer. Data were recalculated from ref. 19.

Com- pound	Methanol concentration (%, v/v)	$k'$			
		Mean	Max.	Min.	S.D. <sup>a</sup>
Phenol	40	2.24	2.80	2.02	0.21
	50	1.26	1.29	1.14	0.10
	60	0.71	0.83	0.75	0.02
	70	0.56	0.57	0.50	0.02
	80	0.34	0.39	0.33	0.02
$S$		2.01	2.07	1.93	0.07
Benzene	40	12.27	13.22	11.38	0.60
	50	6.56	6.99	5.91	0.50
	60	3.55	3.86	3.22	0.15
	70	1.97	2.09	1.75	0.07
	80	1.11	1.21	1.06	0.04
$S$		2.61	2.60	2.59	0.01
Toluene	40	29.83	31.80	27.25	1.07
	50	13.92	16.07	13.32	0.90
	60	7.13	7.81	5.54	0.84
	70	3.24	3.46	2.40	0.28
	80	1.66	1.89	1.56	0.07
$S$		3.14	3.16	3.23	0.05

<sup>a</sup> The standard deviations of the mean of the capacity factors were determined by 20-30 individual measurements at each eluent composition over the 2-year period; the standard deviations of the  $S$  index were determined by only three measurements.

TABLE II

COMPARISON OF LONG-TERM REPRODUCIBILITIES OF  $k'$  AND  $S$  INDEX OVER A 2-YEAR PERIOD WITH ACETONITRILE-WATER AS MOBILE PHASE

Column, Spherisorb  $C_{18}$ ; eluent, acetonitrile-buffer. Data were recalculated from ref. 19.

Com- pound	Acetonitrile concentration (%, v/v)	$k'$			
		Mean	Max.	Min.	S.D. <sup>a</sup>
Phenol	30	2.46	2.48	1.84	0.21
	40	1.51	1.64	1.38	0.07
	50	0.96	1.09	0.84	0.18
	60	0.65	0.68	0.63	0.02
	70	0.44	0.47	0.42	0.01
80	0.31	0.39	0.24	0.05	
$S$		1.79	1.76	1.74	0.03
Benzene	30	13.76	15.38	11.99	1.02
	40	6.90	7.79	6.14	0.57
	50	3.70	4.27	2.98	0.37
	60	2.14	2.28	2.03	0.10
	70	1.31	1.40	1.29	0.05
80	0.84	0.95	0.74	0.06	
$S$		2.42	2.44	2.36	0.04
Toluene	30	29.75	31.98	25.27	2.19
	40	12.78	14.37	11.31	1.20
	50	6.12	7.01	4.94	0.59
	60	3.18	3.41	3.01	0.15
	70	1.87	1.98	1.73	0.08
80	1.17	1.26	1.09	0.09	
$S$		2.83	2.83	2.71	0.07

<sup>a</sup> See Table I.

TABLE III

S VALUES OF EIGHTEEN COMPOUNDS AS A FUNCTION OF THE SURFACE COVERAGE WITH METHANOL–WATER AS MOBILE PHASE

Column, LiChrosorb C<sub>18</sub>; eluent, methanol–water (methanol from 10 to 100%, v/v). Data were recalculated from refs. 17 and 18.

Compound	Coverage (mmol/g)				Mean ± S.D.
	0.255	0.335	0.499	0.690	
Pentane	3.70	3.79	3.67	3.74	3.73 ± 0.05
Hexane	4.30	4.35	4.28	4.26	4.30 ± 0.04
Heptane	4.90	4.92	4.82	4.82	4.87 ± 0.05
Octane	5.47	5.49	5.41	5.43	5.45 ± 0.04
Nonane	6.05	6.08	6.01	6.06	6.05 ± 0.03
Decane	6.66	6.74	6.62	6.62	6.66 ± 0.06
Benzene	2.67	2.64	2.72	2.74	2.69 ± 0.05
Toluene	3.23	3.25	3.23	3.28	3.25 ± 0.03
Ethylbenzene	3.91	3.90	3.92	3.86	3.90 ± 0.03
<i>n</i> -Propylbenzene	4.52	4.54	4.54	4.41	4.50 ± 0.06
<i>n</i> -Butylbenzene	5.07	5.08	5.03	4.87	5.01 ± 0.10
<i>n</i> -Pentylbenzene	5.62	5.69	5.55	5.37	5.56 ± 0.14
<i>n</i> -Hexylbenzene	6.21	6.26	6.14	6.06	6.17 ± 0.09
1-Butanol	1.84	1.94	1.93	1.89	1.90 ± 0.05
1-Pentanol	2.34	2.47	2.47	2.41	2.42 ± 0.06
1-Hexanol	2.92	2.95	2.96	2.93	2.94 ± 0.02
1-Heptanol	3.40	3.50	3.49	3.45	3.46 ± 0.05
1-Octanol	3.93	4.00	4.01	3.98	3.98 ± 0.04
Acetophenone	2.63	2.66	2.77	2.73	2.70 ± 0.06
<i>o</i> -Cresol	2.68	2.78	2.81	2.72	2.75 ± 0.06
Benzyl alcohol	2.27	2.36	2.31	2.33	2.32 ± 0.04
Phenol	2.29	2.31	2.25	2.30	2.29 ± 0.03
Aniline	2.10	2.14	2.11	2.18	2.13 ± 0.04

from the same source but different batches are used. This causes difficulties in using retention data from the literature. It has been observed that the *S* index can eliminate the difference between C<sub>18</sub> packing materials and can act as an important parameter for the standardization of different C<sub>18</sub> packing materials.

Table III gives the *S* values of some compounds on different C<sub>18</sub> packing materials with surface coverages ranging from 0.255 to 0.690 mmol/g with methanol–water as mobile phase. Although a considerable variation in *k'* on the different C<sub>18</sub> bonded phases was observed, the *S* index remains a characteristic constant for a given compound. *S* is independent of the bonded phase density for the solutes

tested. The standard deviations of the *S* index for a specific solute are about 0.05.

The *S* index for a particular solute can make the standardization of different C<sub>18</sub> packing materials possible. The difference between different C<sub>18</sub> bonded phases can be compensated for by using the *S* index, and therefore the retention data with different compositions of the mobile phase on different C<sub>18</sub> packings can be standardized. As *S* value that characterizes the interactions in the mobile phase for a certain solute is independent of different C<sub>18</sub> packing materials with the same mobile phase, which makes it possible to transfer *k'* values from one packing material to another with different compositions of the mobile phase with only one isocratic experiment from which the difference in log *k'*<sub>w</sub> for two kinds of C<sub>18</sub> packing materials can be determined [26]. Therefore, this will allow us to transfer separation results from one C<sub>18</sub> column to another. Details will be given elsewhere.

Tables IV and V give other examples of the *S* index as a function of different column systems with different reversed-phase packing materials.

It can be seen that although *k'* measured with a certain mobile phase composition on different C<sub>18</sub> packing materials varies considerably, the *S* value for a given solute is not affected by the packing material used and reflects the properties of the solute. It is nearly constant even when column systems with different C<sub>18</sub> packing materials are used, which means that the dependence of log *k'* on eluent composition results in parallel lines for a given solute on different reversed-phase packing materials. This parallel behaviour has been observed in many other studies.

Czok and Engelhardt [22] used four constants, *A*, *B*, *C* and *D*, to describe the retention of homologous series in RP-HPLC:

$$\ln k' = A + Bn + CX + DnX \quad (4)$$

where *X* is the concentration of methanol in the mobile phase and *n* is the chain length of a homologous series. Our interest is in the values of *C* and *D*, where *C* shows the change in log *k'* with increasing water content for the basis of the homologous series with *n* = 0. They found that for all reversed-phase packings *C* has about the same values with an average of 5.5 with a relative standard deviation of 6%. Parameter *D* shows the variation of methylene

TABLE IV

EFFECT OF DIFFERENT C<sub>18</sub> PACKING MATERIALS ON S INDEX WITH METHANOL–WATER AS MOBILE PHASE

For experimental conditions, see text. Eluent, methanol–water (methanol from 60 to 90%, v/v).

Compound	C <sub>18</sub> packing <sup>a</sup>						Mean ± S.D.
	1	2	3	4	5	6	
Benzene	2.74	2.68	2.74	2.84	2.81	2.73	2.76 ± 0.06
Toluene	—	—	—	—	3.32	3.24	3.28 ± 0.06
Naphthalene	3.60	3.57	3.70	3.70	3.83	3.69	3.73 ± 0.09
Biphenyl	4.24	4.20	4.36	4.35	4.49	4.30	4.32 ± 0.10
Phenanthrene	4.44	4.43	4.40	4.46	4.69	4.55	4.50 ± 0.11
Anthracene	4.54	4.61	4.49	—	—	—	4.55 ± 0.06
Chrysene	5.24	5.20	5.28	—	—	—	5.24 ± 0.04
<i>p</i> -Terphenyl	5.73	5.68	5.71	—	—	—	5.71 ± 0.03
Anisole	2.74	2.69	2.79	2.85	2.88	2.72	2.78 ± 0.08
Benzyl alcohol	2.11	2.01	2.08	2.18	—	—	2.10 ± 0.07
Benzophenone	2.39	2.29	2.38	—	—	—	2.35 ± 0.06
<i>p</i> -Nitrotoluene	3.05	3.03	3.10	—	—	—	3.06 ± 0.04
<i>n</i> -Butyl benzoate	4.16	4.11	4.20	—	—	—	4.16 ± 0.05

<sup>a</sup> 1 = YWG-C<sub>18</sub>; 2 = ES-C<sub>18</sub>; 3 = Nucleosil-C<sub>18</sub>; 4 = LiChrosorb RP-C<sub>18</sub>; 5 = B4-C<sub>18</sub>; 6 = B8-C<sub>18</sub>.

selectivity with solvent composition, and they found that *D* is also largely constant for all of the packing materials investigated, its average values being 0.88 with a relative standard deviation of 9%.

As *C* and *D* determine the values of *S*, *S* therefore remains constant for all the packings for a specific

solute, and all the stationary phases exhibit parallel behaviour.

In ref. 23, Fig. 6 illustrated the variation of the retention of ethylbenzene with the concentration of water on different alkyl-bonded phases with chain lengths ranging from methyl to octadecyl, and

TABLE V

## EFFECT OF DIFFERENT REVERSED-PHASE PACKING MATERIALS ON S INDEX WITH METHANOL–WATER AS MOBILE PHASE

Data are from ref. 20. Eluent, methanol–water (methanol from 00 to 00%, v/v).

Compound	C <sub>18</sub> packing <sup>a</sup>						Mean ± S.D.
	1	2	3	4	5	6	
Toluene	3.41	3.36	3.72	3.49	3.38	3.34	2.45 ± 0.14
Chlorobenzene	3.52	3.48	3.82	3.62	3.52	3.44	3.57 ± 0.14
Fluorobenzene	3.21	3.20	3.37	3.19	3.19	3.16	3.22 ± 0.08
Benzene	2.97	2.97	3.12	3.07	2.97	2.92	3.00 ± 0.08
Nitrobenzene	2.88	2.85	4.04	2.88	2.99	2.80	2.91 ± 0.09
Benzyl alcohol	2.53	2.57	2.52	2.52	2.53	2.48	2.53 ± 0.03
Phenol	2.55	2.54	2.66	2.55	2.55	2.50	2.56 ± 0.05

<sup>a</sup> 1 = LiChrosorb RP-C<sub>18</sub>; 2 = LiChrosorb RP-C<sub>18</sub>; 3 = LiChrosorb RP-Select B-5; 4 = Kieselgel 60 C<sub>18</sub>; 5 = Biosil-55 RP-18; 6 = Polygosil 60 RP-18.

showed an identical dependence of  $\log k'$  on the eluent concentration [23]. The almost exactly parallel lines on the plot of  $\ln k'$  vs.  $\phi$  demonstrated that the  $S$  index for a specific compound is nearly constant. As an example, for toluene in Fig. 6 in ref. 23  $S = 6.4$  for RP-8 and  $S = 6.6$  for RP-18 and for ethylbenzene  $S = 7.3$  and  $7.5$ , respectively. Braumann [24] also observed this parallel retention behaviour for benzene on six alkyl-bonded packing materials, which demonstrates that  $S$  reflects the property of a given solute for a certain binary mobile phase.

All the results showed that the  $S$  index was determined mainly by the interaction between the solute and the mobile phase. The  $S$  index for a specific solute obtained with the same LC system shows little deviation, but it may diverge slightly from laboratory to laboratory. We compared the  $S$  values given in numerous publications for a similar concentration range of methanol in water for a specific compound. The average standard deviation of the mean  $S$  values is about 0.15.

Factors influencing the  $S$  values may mainly include the homogeneity of the bonded stationary phases, the linearity of the relationship between  $\log k'$  and  $\phi$ , the column temperature and solute structure parameters. The solute structure parameters are one of the most important variables in determining  $S$  values, which will be discussed elsewhere.

The homogeneity of the bonded phases affects the linearity of the  $\log k'$  versus  $\phi$  relationship, which therefore leads to a change in  $S$  values [16,27]. If solute retention is governed by a hydrophobic mechanism, the reproducibility of  $S$  values for a particular solute can be achieved in a given eluent system by using energetically homogeneously distributed bonded phases. The reproducibility of the  $S$  index for a particular solute can serve as a useful parameter for comparison of the energetic homogeneity between different  $C_{18}$  bonded phases. When a compound is extreme polar or ionizable in an aqueous mobile phase, a parabolic shape of the  $\log k'$  versus  $\phi$  plot has been observed [27] and demonstrated different retention mechanisms over the whole concentration range of the mobile phase. This implies an inhomogeneous character of the stationary phase (silanophilic interaction) and of the molecular solute itself (ionic interaction), which means that  $S$  values are difficult to ascertain for these

TABLE VI

EFFECT OF BUFFER ON  $S$  VALUES FOR SOME AROMATIC COMPOUNDS

Column, Hypersil-ODS; eluent, methanol–water. Data are from ref. 25.

Compound	$S^a$	$S^b$
Benzene	2.79	2.77
Chlorobenzene	3.46	3.46
Phenol	2.66	2.59
Benzoic acid	—	3.27
4-Chlorobenzoic acid	—	3.86
4-Chlorotoluene	3.98	3.99
1,4-Dinitrobenzene	2.87	2.80
2-Hydroxypropane	1.37	1.35
2-Nitropropane	1.98	1.94
2-Chloropropane	2.70	2.78

<sup>a</sup> Methanol–water.<sup>b</sup> Methanol–phosphate buffer (pH 2.15).

compounds. Therefore, during the practical separation of ionizable compounds, suppression agents or buffers should be added to the mobile phase to suppress the ionization of these compounds in order to enhance solute–hydrocarbonaceous ligand hydrophobic interactions, and therefore the pH should be strictly controlled when determining  $S$  values for these compounds. The presence of buffer in the mobile phase does not affect the  $S$  values for non-electrolytes, as shown in Table VI.

Temperature plays an important role in RP-HPLC separations. The column temperature should be carefully controlled when determining  $S$  values [24,28,29]. A detailed study of the  $S$  index as a function of column temperature for some non-ionic compounds is summarized in Tables VII and VIII. In the temperature range investigated the  $S$  values were observed to decrease with increasing column temperature, which can be clearly explained by eqn. 3. As the difference in the solute–weak solvent and solute–strong solvent free energy change characterizes the solute interactions in the mobile phase and approaches a constant value for a particular solute with different column temperatures, the value of  $ST$  approaches a constant value for non-ionic compounds, as is demonstrated by the following equation:

$$\Delta G_{\Lambda,C}^0 - \Delta G_{\Lambda,B}^0 = S_1 T_1 = S_2 T_2 = S_3 T_3 \quad (5)$$

TABLE VII

EFFECT OF COLUMN TEMPERATURE ON *S* INDEX FOR SOME POLYCYCLIC AROMATICS WITH METHANOL-WATER AS MOBILE PHASEColumn, Finepak-SIL-C<sub>18</sub>; eluent, methanol-water (methanol from 65 to 80%, v/v). Data were recalculated from ref. 24.

Compound	Column temperature (°C)							
	70		60		50		40	
	<i>S</i>	<i>ST</i>	<i>S</i>	<i>ST</i>	<i>S</i>	<i>ST</i>	<i>S</i>	<i>ST</i>
Naphthalene	2.46	844	2.49	829	2.78	898	2.78	870
Acenaphthylene	2.70	926	2.70	899	3.00	969	3.09	967
Phenanthrene	3.26	1118	3.31	1102	3.68	1189	3.57	1117
Anthracene	3.36	1152	3.43	1142	3.81	1231	3.93	1230
Fluoranthene	3.71	1273	3.60	1199	4.03	1302	4.15	1299
Benzo[ <i>a</i> ]pyrene	4.42	1516	4.40	1465	4.90	1583	5.01	1568

where  $S_1$ ,  $S_2$  and  $S_3$  are *S* values at temperatures  $T_1$ ,  $T_2$  and  $T_3$ , respectively. Therefore, the *S* values decrease with increasing column temperature in order to keep *ST* almost constant for a particular solute, which means that there is a larger solute-water than solute-methanol free-energy change, as it can be seen in Tables VII and VIII.

Tables VII and VIII illustrate the effect of temperature on the *S* index for polycyclic aromatic hydrocarbons and some non-ionic compounds in methanol-water as mobile phase. The values of *ST* for

each compound at different temperatures are also given.

Table IX demonstrates the effect of dead time measurements on the *S* index. As can be seen, the effect of dead time measurements using methanol and sodium nitrite on the *S* index is not obvious, at least for the solutes tested in this experiment.

We conclude that the *S* index is mainly determined by the difference between solute-weak solvent and solute-strong solvent free-energy changes. *S* values are nearly constant even when column

TABLE VIII

EFFECT OF COLUMN TEMPERATURE ON *S* INDEX FOR SOME NON-IONIC COMPOUNDSColumn, C<sub>8</sub>; eluent, methanol-water (methanol from 50 to 70%, v/v). Data are calculated from refs. 28 and 29.

Compound	Column temperature (°C)							
	30		41		51		59.5	
	<i>S</i>	<i>ST</i>	<i>S</i>	<i>ST</i>	<i>S</i>	<i>ST</i>	<i>S</i>	<i>ST</i>
<i>p</i> -Nitrophenol	2.73	827	—	—	2.36	765	2.26	752
Phenol	2.57	779	2.30	722	2.23	723	2.09	695
Acetophenone	2.93	888	2.66	835	2.51	813	2.38	791
Methyl benzoate	3.28	994	3.07	964	2.85	923	2.83	941
Anisole	2.97	900	2.74	860	2.60	842	—	—
Benzene	2.88	873	2.63	826	2.55	826	2.43	808
Phenetole	3.39	1027	3.18	999	3.03	982	2.97	988
Toluene	3.35	1015	3.09	970	2.98	966	2.98	991
Ethylbenzene	3.80	873	3.59	826	3.46	826	3.30	808

TABLE IX  
EFFECT OF DEAD TIME MEASUREMENTS ON  $S$  INDEX

Column, Polygosil-C<sub>18</sub>; eluent, methanol-water. Dead times were measured using sodium nitrite and methanol as a non-retained compound. For experimental conditions, see text.

Compound	Non-retained compound	Methanol concentration (% v/v)			$S$
		0.90	0.80	0.70	
Nitrobenzene	Methanol	0.35	0.64	1.26	2.75
	Sodium nitrite	0.29	0.55	1.08	2.84
Naphthalene	Methanol	0.81	1.80	4.55	3.75
	Sodium nitrite	0.73	1.64	4.12	3.77
Toluene	Methanol	0.61	1.25	2.77	3.29
	Sodium nitrite	0.54	1.12	2.48	3.33
Acenaphthene	Methanol	1.47	3.64	10.33	4.23
	Sodium nitrite	1.36	3.37	9.47	4.21

systems with different packing materials are used.  $S$  varies with the strong solvent used, and hence it represents the solvent strength to the eluent only for a specific solute. A more detailed examination of  $S$  values as a function of molecular structure parameters will be discussed in a further paper.

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