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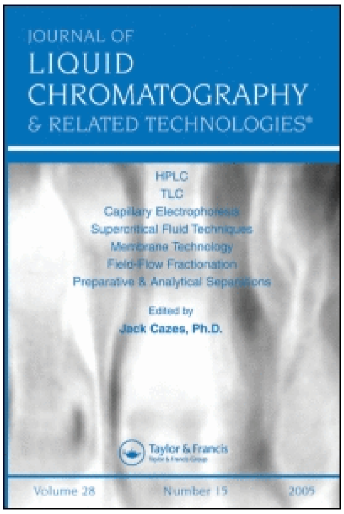

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N. Chen[a]; Y. Zhang[a]; P. Lu[a]
[a] National Chromatographic R. & A. Center, Dalian Institute of Chemical Physics Chinese Academy of Sciences, Dalian, People's Republic of China

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STANDARDIZATION OF DIFFERENT OCTADECYL BONDED PHASES BASED ON THE RETENTION EQUATION IN REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

N. CHEN*, Y. ZHANG, AND P. LU
National Chromatographic R. & A. Center
Dalian Institute of Chemical Physics
Chinese Academy of Sciences
116012 Dalian, People's Republic of China

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ABSTRACT

The method for standardization of different n-octadecyl (C_{18}) bonded phases is discussed based on the retention equation $\log k' = k_w + c\varphi$ in reversed-phase high-performance liquid chromatography (RP-HPLC). The dependence of log k' on eluent composition results in parallel lines for C_{18} reversed phases. The difference in C_{18} bonded phases can be compensated for by using hydrophilic index c which is independent of C_{18} reversed phases for a particular solute. The difference between C_{18} columns packed with C_{18} reversed phases arises from the difference in phase ratio. A linear relationship between parameter k_w and the logarithm of the surface coverage of C_{18} bonded phases has been found. Linear $k_w - k_w$ plots with unit slope on various column pairs have been demonstrated. $k_w - k_w$ plots have

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been utilized to transfer of retention values from one C_{18} column to another over a wide range concentration of mobile phase in RP—HPLC.

INTRODUCTION

The introduction of reversed phases in high — performance liquid chromatography (HPLC) results in the tremendous increase of applications of HPLC [1—4]. It is estimated that somewhere 70% of all HPLC separations are carried out in the reversed phase mode [4]. n—Octadecyl (C_{18}) bonded stationary phases or ODS are now the most widely used column packing material for reversed—phase high—performance liquid chromatography (RP—HPLC), but unfortunately, the standardization of different C_{18} packing materials is still a problem to be solved in RP—HPLC. It is generally observed that the retention values differ when column systems with the same mobile phase concentration but ODS packing materials from different sources or even from the same source but different batches are used. This causes a difficult in using retention data from the literature [5—10]. In some cases, the variation in retention values with the same C_{18} column over a prolonged period even under closely controlled chromatographic conditions is considerable, which makes the standardization of C_{18} bonded phases to be extremely difficult [5]. Therefore, any attempt to use capacity factors alone to compare the results from different laboratories is virtually impossible.

The diversity of octadecyl bonded phases is caused by the different phaysical and chemical properties of the C_{18} reversed phases. There are several parameters that affect retention which could vary with different manufacturing process for C_{18} silica. There have been many studies of the effect of several bonded — phase characteristics, including chain length, carbon content, surface and residual silanols on the retention of a particular solute [1—4]. Systematically investigation on the comparison and characterization of different reversed phase materials could be found in refs. 2—4, in which they have demonstrated that the differences in carbon content can be com-

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pensated for by changing the eluent composition. They have also shown that the dependence of ln k' on eluent composition resulted in parallel lines for all stationary phases in RP—HPLC, which shows us the way to standardize the different C_{18} bonded phases.

In order to make the comparison and standardization of different C_{18} bonded phases over a wide range concentrations of mobile phase, we should first establish the equation to describe the relationship between capacity factors and eluent composition in RP—HPLC. The aim of this work has been to develop a method for transferring of retention values from one ODS packing material to another over a wide range concentrations of mobile phase.

We demonstrated that the difference in C_{18} reversed phases can be compensated for by using hydrophilic index c which is derived from retention equation $\log k' = k_w + c\varphi$ in RP—HPLC. Index, c, of a particular solute is determined mainly by the properties of the mobile phase. It is a constant despite a considerable variation in retention values on different C_{18} columns. Linear $k_w - k_w$ plots on various column pairs for various compounds have been discussed. $k_w - k_w$ plots have been utilized to transfer of capacity factors from one C_{18} column to another over a wide range concentration of the mobile phase in RP—HPLC.

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EXPERIMENTAL

The experimental results utilized in this paper were partly from a paper by Petrovic et al. [7]which give an exact description of the analytical conditions employed. Other HPLC measurements were carried out using a chromatographic system equipped with YSB—2 pump (Shanghai Instrumental Plant, Shanghai, China), Jasco UVIDEC—100—Ⅱ UV detector (Japan Spectroscopic Co., Ltd., Tokyo, Japan)operating at 254nm, the YWG—C_{18}(Tinjing Chemical Reagent Factory, Tinjing, China), ES—C_{18}(ES Industry Inc., USA), Nucleosil—10RP—C_{18}, Lichrosorb—RP—C_{18} as the stationary phases in a 125 * 4. 6 mm I. D.. The mobile phase were prepared volumetrically from individually measured aliquots of methanol and water; the flow rate was set at 0. 9 ml/min. The column dead time, t0, was determined using the sodium nitrite as the non—retained compound. All measurements were performed at room temperature.

RESULTS AND DISCUSSION

In order to make the standardization of different C_{18}—bonded phases over a wide range concentration of mobile phase, we should first illustrate the basic retention equation to describe the effect of eluent composition on retention in RP—HPLC.

The most popular description of the retention behaviour is the linear approximation of the relationship between logarithm of capacity factor (log k') and the volume fraction of the organic modifier (φ) [3,6,11—12], as shown by the equation (1a). This equation has obtained considerable application in practical RP—HPLC and has found no significant errors in practical uses, this fundamental retention equation could be derived by using physicochemical method with combination of empirical relation [8].

$$\mathrm{lok}\ k' = k_w + c\varphi \qquad (1a)$$

Equation (1a) can often be written as:

$$\log k' = \log k'_w - S\varphi \qquad (1b)$$

The physicochemical meanings of parameters c (or —S) and k_w(or $\log k'_w$) can be expressed as follows:

$$k_w = \log\beta + (\triangle G^0_{A,C} - \triangle G^0_{A,L})/RT \qquad (2)$$

$$c = (\triangle G^0_{A,B} - \triangle G^0_{A,C})/RT \qquad (3)$$

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where RT is the gas constant multiplied by absolute column temperature, β refers to the phase ratio, subscripts A, B, C refer to solute, strong solvent and weak solvent, respectively, $\Delta G^0_{A,B}$ and $\Delta G^0_{A,C}$ are the mon—electrostatic free—energy change of solute—strong sovent and solute—weak solvent, respectively. $\Delta G^0_{A,L}$ is the non—electrostatic free—energy change of solute—hydrocarbonaceous ligands.

Table I shows the parameters k_w and c on C_{18} reversed phases with their surface coverages ranging from 0. 255 to 0. 690 mmol/g with water—methanol as mobile phase for various compounds.

we have also measured the capacity factors of twelve solutes with different C_{18} stationary phases over methanol concentration ranging from 0. 9 to 0. 5. Table I summarized the results of the correlation of log k' and φ with various solutes on different C_{18} packing materials.

The results in these Tables indicated that despite a considerable variations in the retention values on different stationary phases, parameter, c, of a particular compound on different C_{18} columns remains constants. The data agree quite well with our theoretical assumptions. As shown in equation (3), parameter c which is defined as the hydrophilic index quantitatively describes the difference between solute—strong solvent and solute—weak solvent interactions at a certain column temperature. Therefore parameter c for a specific solute is a constant even when the column systems with the different C_{18} packings are used. It is independent of different C_{18} packings, which is consistent with the results obtained by Engelhardt el al. 〔3—4〕. They have demonstrated that the dependence of lnk' on eluent composition results in parallel lines for all stationary phases.

Therefore the difference in different C_{18} bonded phases can be compensated for by using hydrophilic index c, it characterizes the properties of the mobile phase. It paves the way for the standardization of different C_{18} reversed phases. Parameter k_w which is defined as the hydrophobic index is the logarithm of an extrapolated value of the capacity factor in pure water. The values of k_w are usually too high to obtain experimentally and therefore have to be calculated using extrapolation techniques. Parameter k_w contains not

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Table I.

Parameters k_w and c on the C_{18} reversed phases with the surface coverage ranging from 0.255 to 0.690 mmol/g with methanol water as mobile phase for various compounds .*

The experimental data are recalculated from ref. 7

		Coverage (mmol/g)							
		0.225		0.335		0.499		0.690	
Code	solute	kw(Ⅰ)	c	kw(Ⅱ)	c	kw(Ⅲ)	c	kw(Ⅳ)	c
1	Pentane	2.72	−3.70	2.93	−3.79	3.07	−3.67	3.21	−3.74
2	Hexane	3.40	−4.30	3.58	−4.35	3.74	−4.28	3.89	−4.26
3	Heptane	4.08	−4.90	4.24	−4.92	4.37	−4.82	4.56	−4.82
4	Octane	4.74	−5.47	4.89	−5.49	5.04	−5.41	5.21	−5.43
5	Nonane	5.42	−6.05	5.56	−6.08	5.71	−6.01	5.87	−6.06
6	Decane	6.10	−6.66	6.27	−6.74	6.39	−6.62	6.52	−6.62
7	Benzene	1.48	−2.67	1.64	−2.64	1.88	−2.72	2.05	−2.74
8	Toluene	2.15	−3.23	2.30	−3.25	2.54	−3.23	2.71	−3.28
9	Ethylbenzene	2.87	−3.91	3.02	−3.90	3.25	−3.92	3.42	−3.86
10	n−Propylbenzene	3.54	−4.52	3.68	−4.54	3.91	−4.54	4.07	−4.41
11	n−Butylbenzene	4.20	−5.07	4.33	−5.08	4.56	−5.03	4.72	−4.87
12	n−Pentylbenzene	4.82	−5.62	4.94	−5.69	5.17	−5.55	5.34	−5.37
13	n−Hexylbenzene	5.48	−6.21	5.59	−6.26	5.83	−6.14	5.98	−6.06
14	Butanol−1	0.57	−1.84	0.72	−1.94	0.93	−1.93	1.09	−1.89
15	Pentanol−1	1.19	−2.34	1.33	−2.47	1.54	−2.47	1.71	−2.41
16	Hexanol−1	1.80	−2.92	1.94	−2.95	2.14	−2.96	2.31	−2.93
17	Heptanol−1	2.41	−3.40	2.55	−3.50	2.75	−3.49	2.91	−3.45
18	Octanol−1	3.03	−3.93	3.16	−4.00	3.36	−4.01	3.52	−3.98

* column: Lichrosorb−C_{18}; eluent: methanol−water.

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Table Ⅱ.

Parameters k_w and c with different C_{18} packings with water—methanol as mobile phase for various compounds.*

		column 1		column 2		column 3		column 4	
Code	solute	k_w	—c	k_w	—c	k_w	—c	k_w	—c
1	Benzene	2.05	2.74	2.03	2.68	2.24	2.74	2.74	2.84
2	Naphthalene	3.03	3.60	3.07	3.57	3.34	3.70	3.41	3.70
3	Biphenyl	3.70	4.24	3.72	4.20	4.03	4.36	4.13	4.35
4	Phenanthrene	4.04	4.44	4.10	4.43	4.26	4.40	4.44	4.46
5	Anthracene	4.17	4.54	4.29	4.61	4.39	4.49		
6	Chrysene	5.06	5.24	5.10	5.20	5.39	5.28		
7	p—Terphenyl	5.40	5.73	5.45	5.68	5.66	5.71		
8	Anisole	2.04	2.74	2.04	2.69	2.27	2.79	2.39	2.85
9	Benzyl alcohol	1.16	2.11	1.07	2.01	1.25	2.08	1.36	2.18
10	Acetophenone	1.62	2.39	1.50	2.29	1.72	2.38		
11	p—Nitrotoluene	2.32	3.05	2.36	3.03	2.57	3.10		
12	n—Butyl benzoate	3.55	4.16	3.58	4.11	3.82	4.20		

* Column 1: YWG—C_{18}, column 2: ES—C_{18}, column 3: Nucleosil—10RP—C_{18}, column 4: Lichrosorb—RP—C_{18}; eluent: methanol—water.

only the phase ratio of the column but also the difference in hydrophobic interaction between solute—water and solute—bonded phases. It characterizes the C_{18} column for a specific solute.

As shown in Table I, the values of k_w are increasing with the surface coverages of C_{18} packings. This can be explained theoretically. As the phase ratio is defined as the ratio of the volume of the bonded C_{18} ligands to the volume of the mobile phase. It can be described as follows:

$$\beta = V_{C18} * N_{C18} * W/V_m \qquad (4)$$

where N_{C18} is the numbers of moles of C_{18} bonded ligands, V_{C18} is the molar volume of C_{18} per unit mass of column packings. W is the mass of the column packing. V_m refers to the volume of the mobile phase. Therefore k_w can be expressed as follows:

$$k_w = \log[V_{C18} * W/V_m] + \log N_{C18} + (\triangle G^0_{A,C} - \triangle G^0_{A,L})/RT \qquad (5)$$

Parameter k_w has a linear relationship with the logarithm of surface coverages of C_{18} packings with unit slope. The linear correlation of k_w with the logarithm of the surface coverage of C_{18} bonded phases is demonstrated by Figure 1.

Therefore, the variations observed in retention behavior with different C_{18} reversed phases arise from differences in phase ratios, which therefore results in linear k_w—k_w plots on various C_{18} column pairs (see Figures 2 and 3).

Therefore, under the condition that there is no alteration of solute configuration during the interaction with the hydrocarbonaceous ligands, plots of k_w values obtained on one C_{18} stationary phase, k_w(I), versus those obtained on another, k_w(II), with the same eluent system can be written as follows:

$$k_w(\mathrm{I}) = K_1 * k_w(\mathrm{II}) + K_2 \qquad (6)$$

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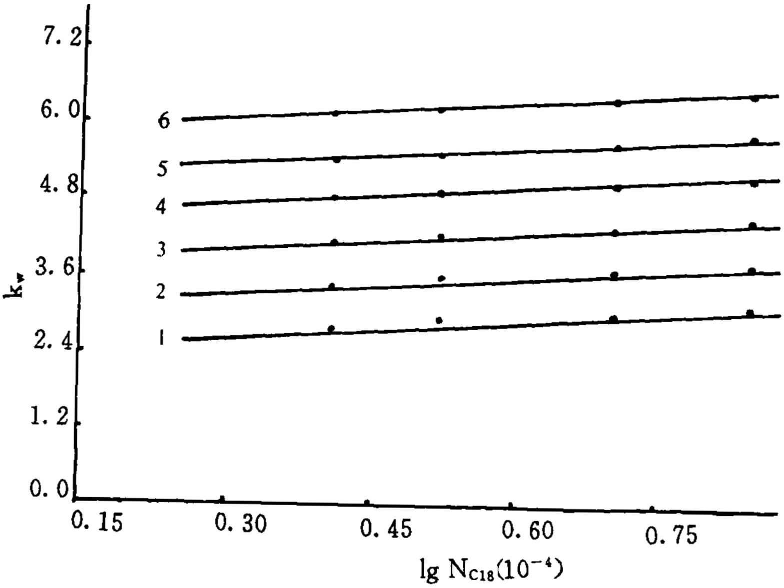

Figure 1. Linear relationship between k_w and the logarithm of surface coverage of C_{18} bonded phases; for chromatographic conditions see Table I ; 1: pentane, 2: hexane, 3: heptane, 4: octane, 5: nonane, 6: decane.

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where K_1 is the slope of the $k_w - k_w$ plots. $k_w - k_w$ plots usually have unit slope. K_2 is the difference in k_w values between C_{18} columns. Figure 2 demonstrates linear $k_w - k_w$ plots on various C_{18} column pairs. Linear relationship between $k_w(\mathrm{I})$ and $k_w(\mathrm{I})$ in Fig. 2 is obtained by regression analysis as follows:

$$k_w(\mathrm{I}) = 0.998\, k_w(\mathrm{I}) + 0.16, N = 18, R = 0.999 \qquad (7)$$

where N is the number of compounds in the regression analysis, R refers to the correlation coefficient.

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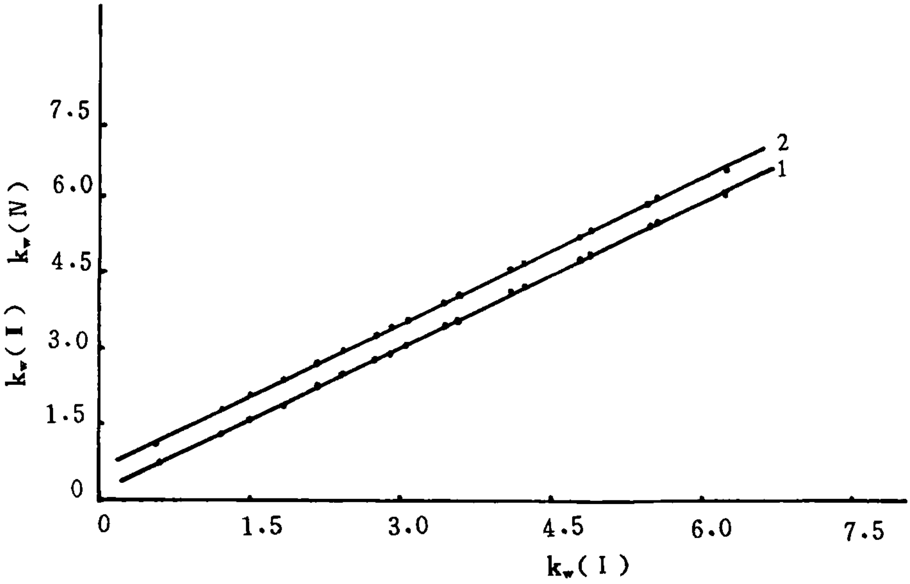

Figure 2. The linear $k_w - k_w$ plots on various column pairs for eighteen compounds; for the chromatographic conditions see Table I ;1:k_w(II) versus k_w(I),2:k_w(IV) versus k_w(I).

Linear $k_w - k_w$ correlation with unit slope indicates identical thermodynamic behavior for two columns. The unit slope of $k_w - k_w$ plots suggests that the retention of solutes on one C_{18} bonded phase over a wide range eluent composition is energetically homogeneous with those on another C_{18}. $k_w - k_w$ plots on various column pairs can serve as a useful tool for comparing the energetical homogeneity between different C_{18} columns. Therefore, for energetically homogeneous C_{18} packings, it is concluded that the main source of the differences between columns packed with octadecyl bonded phases is a difference in parameter k_w.

Table II shows the difference in k_w—values between C_{18} columns for various compounds. As is seen from the Table, the differences in k_w between

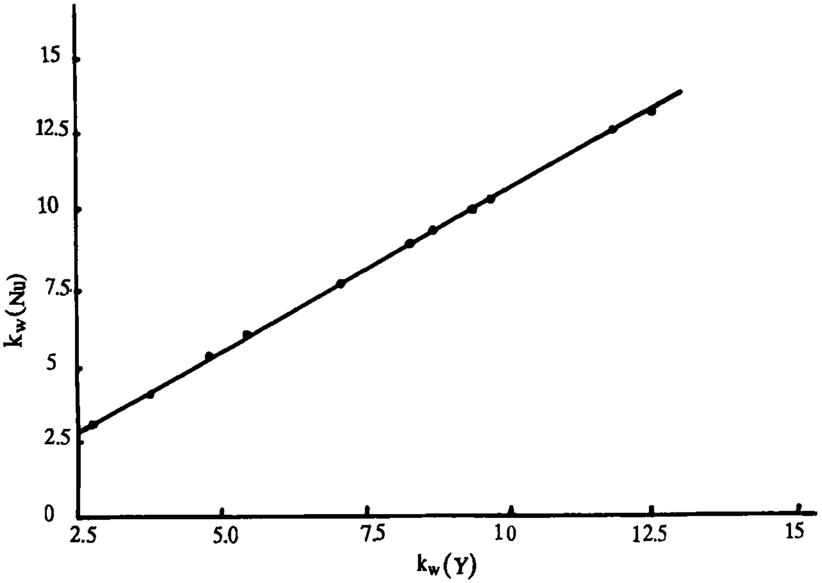

Figure 3. Plot of k_w—values obtained on Nucleosil—10RP—C_{18}, k_w (Nu), versus those obtained on YWG—C_{18}, k_w(Y), for twelve compounds; for chromatographic conditions see Table I.

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two columns is a constant even when different compounds are used. The calculated k_w by using linear k_w—k_w plots on column pairs are also listed in the Table. As seen from the Table, there is a better coincidence between the extrapolated k_w—values and the calculated values.

k_w—k_w plots on various column pairs enable us to transfer of retention data from one ODS packing material to another over a widely range concentration of the mobile phase, which makes the standardization of different C_{18} reversed phases possible.

Table IV shows the results for transferring of the capacity factors of twelve compounds from YWG—C_{18} to Nucleosil—10RP—C_{18}, where the constant c for both materials is considered to be the same. The calculated parameter

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Table III.
Comparison of the extrapolated parameter k_w on different C_{18} bonded phases with those calculated with linear $k_w - k_w$ plots on column pairs.*

	Coverage (mmol/g) 0.255	0.335	0.499	0.690						
Code	k_w(I) exp.	k_w(II) exp.	k_w(III) exp.	k_w(IV) exp.	$\triangle_1$	$\triangle_2$	$\triangle_3$	k_w(II) cal.	k_w(III) cal.	k_w(IV) cal.
1	2.72	2.93	3.07	3.21	0.21	0.35	0.49	2.87	3.07	3.23
2	3.40	3.58	3.74	3.89	0.18	0.34	0.49	3.55	3.74	3.90
3	4.08	4.24	4.37	4.56	0.16	0.29	0.48	4.23	4.41	4.57
4	4.74	4.89	5.04	5.21	0.15	0.30	0.47	4.89	5.07	5.22
5	5.42	5.56	5.71	5.87	0.14	0.29	0.45	5.56	5.74	5.89
6	6.10	6.27	6.39	6.52	0.17	0.29	p.42	6.24	6.41	6.56
7	1.48	1.64	1.88	2.05	0.16	0.40	0.57	1.63	1.85	2.01
8	2.15	2.30	2.54	2.71	0.15	0.39	0.56	2.30	2.51	2.67
9	2.87	3.02	3.25	3.42	0.15	0.38	0.55	3.02	3.22	2.38
10	3.54	3.68	3.91	4.07	0.14	0.37	0.53	3.69	3.88	4.04
11	4.20	4.33	4.56	4.72	0.13	0.36	0.52	4.35	4.53	4.69
12	4.82	4.94	5.17	5.34	0.12	0.35	0.52	4.97	5.15	5.30
13	5.48	5.59	5.83	5.98	0.11	0.35	0.50	5.62	5.80	5.95
14	0.57	0.72	0.93	1.09	0.15	0.36	0.52	0.73	0.95	1.12
15	1.19	1.33	1.54	1.71	0.14	0.35	0.52	1.34	1.56	1.73
16	1.80	1.94	2.14	2.31	0.14	0.34	0.51	1.95	2.16	2.33
17	2.41	2.55	2.75	2.91	0.14	0.34	0.50	2.56	2.77	2.93
18	3.03	3.16	3.36	3.52	0.13	0.33	0.49	3.18	3.38	3.54

* For the chromatographic conditions and code see Table I;
$\triangle_1 = k_w(\text{II}) - k_w(\text{I})$; $\triangle_2 = k_w(\text{III}) - k_w(\text{I})$; $\triangle_3 = k_w(\text{IV}) - k_w(\text{I})$.

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Table Ⅳ.
The calculated and experimental values of parameter k_w and comparison of capacity factors measured on Nucleosil−10RP−C_{18} with the calculated ones for twelve compounds at different concentrations of methanol *.

Code	−c	k_w exp.	k_w cal.		Methanol concentration (%) 95	90	85	80	70	60	50
1	2.74	2.24	2.24	e	0.45	0.59	0.79	1.08	1.99	3.94	7.60
				p	0.43	0.59	0.81	1.12	2.10	3.94	7.41
2	3.60	3.34	3.26	e	0.72	1.02	1.51	2.29	5.29	14.16	
				p	0.69	1.05	1.58	2.40	5.49	12.59	
3	4.24	4.03	3.95	e	0.85	1.29	2.03	3.40	9.24	27.91	
				p	0.84	1.36	2.22	3.61	9.59	25.47	
4	4.44	4.26	4.31	e	1.26	1.94	3.17	5.40	15.58		
				p	1.24	2.06	3.43	5.73	15.92		
5	4.54	4.39	4.44	e	1.32	2.08	3.47	5.91	17.20		
				p	1.34	2.26	3.81	6.43	18.28		
6	5.24	5.39	5.37	e	2.48	4.20	7.42	14.55	50.47		
				p	2.47	4.51	8.24	15.07	50.35		
7	5.73	5.66	5.72	e	1.79	3.22	6.12	12.91			
				p	1.89	3.66	7.07	13.68			
8	2.74	2.27	2.23	e	0.45	0.58	0.77	1.06	2.02	3.82	8.06
				p	0.42	0.58	0.80	1.09	2.05	3.86	7.25
9	2.11	1.25	1.31	e	0.21	0.25	0.30	0.38	0.62	1.00	1.76
				p	0.20	0.26	0.33	0.42	0.68	1.11	1.80
10	2.39	1.72	1.79	e	0.33	0.39	0.48	0.63	1.09	1.93	3.76
				p	0.33	0.43	0.57	0.76	1.31	2.27	3.94
11	3.05	2.57	2.52	e	0.45	0.61	0.81	1.18	2.36	5.06	11.0
				p	0.42	0.60	0.85	1.20	2.43	4.90	9.89
12	4.16	3.82	3.80	e	0.73	1.08	1.69	2.69	7.26	21.23	
				p	0.71	1.14	1.84	2.96	7.73	20.14	

* For chromatographic conditions and code see Table Ⅰ; e denotes the experimental k'; p denotes the predicted ones.

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k_w were determined by linear $k_w - k_w$ plot between these two ODS packing materials.

Figure 3 shows the parameter k_w of 12 compounds on Nucleosil—10RP—C_{18}, k_w(Nu), plotted as a function of k_w on YWG—C_{18}, k_w(Y). A linear $k_w - k_w$ relationship on these two C_{18} columns has been observed.

Retention transferring between different C_{18} columns could be carried out with the establishment of the data base of k_w and c index on the standard C_{18} column. Further studies are needed.

CONCLUSIONS

The different in C_{18} reversed phases can be compensated for by using c index. Linear $k_w - k_w$ plots on various column pairs are observed. $k_w - k_w$ plots are used to transfer of retention values from one C_{18} column to another over a wide range eluent composition.

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