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Method for characterization of selectivity in reversed-phase liquid chromatography

V. Calibration of the retention scale for chromatographic systems with low concentrations of organic solvents in the mobile phase

PAVEL JANDERA* and JITKA ROZKOŠNÁ

Institute of Chemical Technology, Department of Analytical Chemistry, Nám. Legií 565, 53210 Pardubice (Czechoslovakia)

ABSTRACT

The method for the characterization and prediction of selectivity and absolute retention in reversed-phase liquid chromatography based on two indices (a lipophilic and a polar one) has been previously applied to chromatographic systems with mobile phases containing 50% and more of one or two organic solvent(s). Because of a high retention, the homologues series of *n*-alkylbenzenes is not suitable for the direct calibration of the retention scale in mobile phases with lower concentrations of organic solvents, and the extrapolation of the calibration parameters based on the data for this series in mobile phases with low water content yields predicted retention data that are subject to significant errors. To circumvent this inconvenience and to expand the application possibilities of the above method to mobile phases with higher concentrations of water, three homologous series were tested as potential candidates for calibration standards: 3-*n*-alkyl-6-methyluracils, 3-alkoxycarbonyl-2-pyrazolines and alkan-2-ones. All three homologous series tested were found to be suitable substitutes for *n*-alkylbenzenes as calibration standards in mobile phases containing 25–50% methanol in water. The selection of suitable calibration standards for acetonitrile–water mobile phases is more difficult because of a curvature of the dependence of log k' on the concentration of acetonitrile at low concentrations of this solvent in aqueous mobile phases. Here, the alkoxycarbonyl pyrazolines and alkan-2-ones proved useful as calibration standards in mobile phases containing 30–50% acetonitrile. The agreement between the predicted and experimental k' was within 10% or better of the experimental k'.

INTRODUCTION

Because of the restricted availability of a number of physico-chemical constants involved in various theoretical models of retention in reversed-phase liquid chromatography, precise prediction of retention using these complex models is not possible at present without preliminary experimental data. Hence, most prediction methods rely on calibration of the retention scale using suitable reference data. Some prediction methods are based on correlations of the retention data with various structur-

al descriptors and related parameters; other use a scale of retention indices similar to the Kováts retention indices in gas chromatography, based on alkyl aryl ketones [1,2], alkan-2-ones [3,4] or polyaromatic hydrocarbons [5,6] as the reference compounds. Because of various specific solute–solvent interactions, the retention indices are usually sensitive to changes in the composition of the mobile phase [7], and the single-index approach is reliable only within a narrow composition range of mobile phases. The prediction method based on the interaction indices [8,9] also cannot take full account of these effects over a broad range of mobile phase compositions [10].

To avoid the necessity of recalibration of the retention scale with changing mobile phase composition, the retention indices or their structural contributions were expressed as a quadratic function of the concentration of the organic solvent in the mobile phase, and it was suggested that the coefficients of these equations be used in the predictive calculations [7].

Another solution to this problem was introduced earlier. In this, two indices are used simultaneously to characterize the retention of each sample solute [10]. One index, n_{ce}, accounts for the hydrophobicity of the solute matrix, and the other, q_i, for the polarity of the functional groups in the molecule of the solute and its effects on the interactions with the components of the mobile phase. The retention scale is calibrated using a reference homologous series over a wide range of mobile phase compositions and the retention, expressed in terms of capacity factors, k', is predicted using eqn. 1:

$$\log k' = (a_0 + a_1 \cdot n_{ce}) \cdot (1 - p \cdot x) - q_i \cdot x \tag{1}$$

where n_{ce} and q_i are the lipophilic and polar indices of a given solute, respectively, x is the concentration of the organic solvent in the water–organic solvent mobile phase [in % (v/v) $\cdot 10^{-2}$] and a_0, a_1 and p are the constants of the calibration equation, valid for a given type of column packing (stationary phase), organic solvent and calibration homologous series [10]. The index n_{ce} has the meaning of a hypothetical equivalent number of carbon atoms in the alkyl chain of the calibration homologous series and depends on the type of the calibration series; ideally, it should depend only slightly on the column packing material and on the type of organic solvent in the mobile phase. On the other hand, the index q_i is a measure of solute–solvent interactions and is expected to depend strongly on the organic solvent.

Homologous n-alkylbenzenes were found to be suitable for the calibration of the retention scale using eqn. 1 in binary mobile phases containing 50–90% methanol, acetonitrile, 1,4-dioxane or tetrahydrofuran [10,11] and in ternary mobile phases of methanol–acetonitrile–water [12], and the use of this method was later adapted also for reversed-phase chromatography with gradient elution [13].

The indices n_{ce} and q_i can be calculated from the data plot of log k' versus x, using linear regression. It is also possible to use the quadratic regression for the analysis of strongly curved data plots; in this case an additional second-order term with respect to x is introduced in eqn. 1 [10,11]. The constants a_0, a_1 and p are determined from the experimental data of the dependence of k' of the calibration homologous series on x and on the number of carbon atoms in the alkyl chain, n_c, of the individual homologues [14]:

$$\log k' = (a_0 + a_1 \cdot n_c) \cdot (1 - p \cdot x) - q \cdot x \tag{2}$$

The accuracy of the prediction of k' is significantly improved when the differential indices, Δn_c and Δq, related to a standard reference compound (such as toluene, the alkylbenzene with $n_c = 1$) are used instead of n_{ce} and q_i in eqn. 1 to calculate the relative retention with respect to the standard:

$$\alpha = k'/k'_s \tag{3}$$

Eqn. 1 is then transformed to:

$$\log \alpha = a_1 \cdot (1 - p \cdot x) \cdot \Delta n_c - x \cdot \Delta q \tag{4}$$

where $\Delta n_c = n_{ce} - n_{c,s}$ $\Delta q = q_i - q_s$ (5a and b)

and k'_s, $n_{c,s}$ and q_s are the capacity factor k' and the indices n_{ce} and q_i of the reference standard compound (e.g. toluene) [11,12].

This calibration method was found to be useful in explaining the experimental behaviour of various oligomeric series, including occasionally occurring changes in the order of elution depending on the composition of the mobile phase and on the type of the functional end group [15].

In mobile phases containing low concentrations of organic solvent, even the lowest homologous alkylbenzenes are strongly retained ($k' > 10$), which makes the use of the alkylbenzene calibration series unpractical. To allow the use of the above method of calibration and prediction of retention in water-rich mobile phases, another suitable less retained calibration homologous series should be found with less bulk and/or a more polar functional end group than the benzene ring. For this purpose, three homologous series are tested in this work, namely 3-n-alkyl-6-methyluracils, 3-alkoxycarbonyl-2-pyrazolines and alkan-2-ones, all of which are UV-absorbing and thus can be readily used in reversed-phase chromatography with UV detection.

Earlier attempts at using the parameters a_1 and p of the alkylbenzene homologous series, determined in water poor mobile phases, in mobile phases with low concentrations of organic solvents were not successful. Instead of using the extrapolated constants, we decided to measure a few experimental data for toluene, ethylbenzene and, possibly, for n-propylbenzene in the investigated range of mobile phase compositions to determine the constants of the calibration eqn. 4, even though the values determined from a small number of experimental data may not be very precise, in order to get some idea of the performance of the other potential calibration homologous series in comparison with alkylbenzenes.

For the test solutes, we selected some simple aromatic compounds and some structurally more complex pesticides and their metabolites of practical importance.

EXPERIMENTAL

The equiment used included a Model 6000A pump, a U6K injector and a Model 440 UV detector, operated at 254 nm, all from Waters–Millipore (Milford, MA, USA). The detector signal was registered using a TZ 4100 line recorder (Laboratory Instrument Works, Prague, Czechoslovakia). The chromatographic column was stainless steel, 300 × 3.8 mm I.D., packed in the laboratory with Silasorb C_{18},

10 μm, octadecylsilica (Lachema, Brno, Czechoslovakia), using a high-pressure slurry-packing technique.

The mobile phases were prepared by mixing water (double-distilled in glass with addition of potassium permanganate) with either methanol or acetonitrile (spectroscopic grade, Lachema) in desired volume ratios. All the solvents were filtrated using a Millipore 0.45-μm filter and the premixed mobile phases were degassed by ultrasonication before use.

The column dead volume, V_M, was determined as the retention volume of ^2H_2O in each mobile phase employed, using a Model R 401 refractometric detector (Waters). The experimental values of V_M were in the range from 2.36 to 2.25 cm^3 in 25–50% methanol and from 2.64 to 2.05 cm^3 in 25–50% acetonitrile.

The capacity factors, $k' = (V_R/V_M - 1)$, were calculated from the arithmetic means of two or three experimental retention volumes, V_R, in repeated experiments. From the plots of the experimental k' at different compositions of the mobile phase, the parameters a and m of eqn. 6 were determined using linear regression (Table II):

$$\log k' = a - m \cdot x \tag{6}$$

The constants a_0, a_1 and m of the homologous series tested were determined from the constants a and m of the individual homologoues as described elsewhere [11]. The indices n_{ce} and q_i were calculated after combining eqns. 1 and 6 as:

$$n_{ce} = (a - a_0) a_1 \tag{7}$$

$$q_i = m - p \cdot a \tag{8}$$

where a and m relate to the individual test solutes and a_0, a_1 and p to the calibration homologous series. Eqn. 5a and b was used to calculate the indices Δn_c and Δq, related to toluene as the reference compound [11].

The capacity factors of the test solutes were predicted from their differential indices Δn_c, Δq, using eqns. 3 and 4 for each of the calibration series tested.

The sample solutes were purchased or obtained from different sources or synthesized at the Departments of Organic Chemistry and Analytical Chemistry, Institute of Chemical Technology, Pardubice, Czechoslovakia. n-Alkyl-3-alkoxycarbonyl-2-pyrazolines were prepared by derivatization of alkyl acrylates with diazomethane, as described previously [16]. Table I lists the chromatographed homologous and test compounds.

RESULTS AND DISCUSSION

As is demonstrated by the values of the correlation coefficients in Table II, most compounds studied show good linearity of the log k' *versus* methanol concentrations plots in the concentration range investigated, *i.e.* 25–50% methanol in water, in agreement with eqn. 6. In acetonitrile–water mobile phases, the correlation coefficients are lower than in methanol–water mobile phases for some compounds, such as homologous alkyluracils and alkoxycarbonylpyrazolines, aromatic amides and phenylureas with methoxy substituents on both the nitrogen and the benzene ring. This

TABLE I

LIST OF THE CALIBRATION HOMOLOGOUS SERIES AND TEST COMPOUNDS

I. Calibration compounds
(A) n-Alkylbenzenes

(1)	Methylbenzene
(2)	Ethylbenze
(3)	*n*-Propylbenzene

(B) 3-n-Alkyl-6-methyluracils

(4)	3,6-Dimethyluracil
(5)	3-Ethyl-6-methyluracil
(6)	3-*n*-Propyl-6-methyluracil
(7)	3-*n*-Butyl-6-methyluracil

(C) 3-Alkoxycarbonyl-2-pyrazolines

(8)	3-Methoxycarbonyl-2-pyrazoline
(9)	3-Ethoxycarbonyl-2-pyrazoline
(10)	3-*n*-Propyloxycarbonyl-2-pyrazoline
(11)	3-*n*-Butyloxycarbonyl-2-pyrazoline
(12)	3-*n*-Pentyloxycarbonyl-2-pyrazoline

(D) Alkan-2-ones

(13)	Acetone
(14)	Methyl *n*-propyl ketone
(16)	Methyl *n*-butyl ketone

II. Test compounds

(17)	Benzonitrile
(18)	Anisole
(19)	Acetophenone
(20)	Nitrobenzene
(21)	Phenetole
(22)	Phenyl acetate
(23)	Methyl benzoate
(24)	Ethyl benzoate
(25)	Benzaldehyde
(26)	Benzylalcohol
(27)	Aniline
(28)	Phenol
(29)	*o*-Cresol
(30)	*p*-Cresol
(31)	Linuron (N-(3,4-dichlorophenyl)-N'-methoxy-N'-methylurea)
(32)	Ethyl phenylcarbaminate
(33)	Benzene
(34)	*m*-Bromonitrobenzene
(35)	Benzamide
(36)	N-Methylbenzamide
(37)	Phenylurea
(38)	Phenylsulphamide
(39)	N,N-Dimethylbenzamide
(40)	N-Methylphenylsulphamide
(41)	Metoxuron (N-(3-chloro-4-methoxyphenyl)-N',N'-dimethylurea)
(42)	Deschlorometoxuron (N-(4-methoxyphenyl)-N'N'-dimethylurea)
(43)	Hydroxymetoxuron (N-(3-chloro-4-hydroxyphenyl)-N',N'-dimethylurea)
(44)	Methomyl (1-methylthio-O-(N-methylcarbamoyl)acetaldoxime)
(45)	Simazine (2-chloro-4,6-bis-ethylamino-1,3-5-triazine)
(46)	Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine)

behaviour obviously originates in the curvature of the log k' *versus* acetonitrile concentration plots in mobile phases containing 25% acetonitrile. The dependence of log k' on the concentration of acetonitrile in the range 30–50% is fairly linear, as in water–methanol mobile phases.

Table III lists the constants a_0, a_1, p and q of eqn. 2 for the four potential calibration homologous series, determined by linear regression of the log k' data in dependence on both n_c and x. Prior to the determination of these constants, good linearity of the plots of log k' and of the constants a and m (eqn. 6) *versus* the number of carbon atoms in the individual homologues, n_c was verified (the results are not shown here). The values of a_0, a_1, p and q for the homologous n-alkylbenzenes may be subject to errors resulting from the strong retention of higher homologues in mobile phases with lower contents of organic solvent, which did not allow measurement of their retention data. Only the capacity factors of toluene could be determined over the full concentration range of the mobile phases investigated, with the aim of calculating the constants of eqn. 2; the additional data used for this purpose included only k' of n-propylbenzene in 35%-50% acetonitrile (three data points), and k' of n-butylbenzene in 40 and 50% acetonitrile; in water–methanol mobile phases only the k' of n-propylbenzene in 40 and 50% methanol was used.

TABLE II

CONSTANTS a AND m OF EQN. 6 FOR THE CALIBRATION HOMOLOGOUS COMPOUNDS AND TEST SOLUTES IN METHANOL–WATER (I) AND ACETONITRILE–WATER (II) MOBILE PHASES

R = correlation coefficient. The numbers of the solutes are as in Table I.

Solute	I			II		
	a	m	R	a	m	R
n-Alkylbenzenes						
1	2.547	2.917	0.9932	2.558	3.749	0.9932
2	3.102	3.441	[a]	2.837	3.844	0.9967
3	–	–	–	3.149	3.985	[a]
3-n-Alkyl-6-methyluracils						
4	0.425	2.688	0.9999	− 0.023	2.419	0.9535
5	0.824	2.762	0.9990	0.258	2.527	0.9867
6	1.365	3.176	0.9989	0.512	2.538	0.9759
7	1.984	3.699	0.9998	0.904	2.824	0.9975
3-Alkoxycarbonyl-2-pyrazolines						
8	0.624	2.631	0.9967	0.155	1.604	0.9182
9	1.048	2.729	0.998	0.478	1.894	0.9928
10	1.673	3.240	0.9999	0.944	2.427	0.9936
11	2.319	3.825	0.9994	1.513	3.076	0.9970
12	2.877	4.250	[a]	2.012	3.589	0.9936
Alkan-2-ones						
13	0.158	1.688	0.9985	− 0.075	0.851	0.9640
14	0.675	1.951	0.9964	0.505	1.583	0.9983
15	1.232	2.352	0.9982	0.940	1.976	0.9952
16	1.884	2.948	0.9995	1.513	2.691	0.9987

TABLE II (*continued*)

Solute	I			II		
	a	*m*	*R*	*a*	*m*	*R*
Test solutes						
17	1.790	3.022	0.9970	1.630	2.905	0.9972
18	2.199	2.935	0.9981	2.064	3.311	0.9977
19	2.005	3.265	0.9986	1.493	2.702	0.9985
20	1.919	2.848	0.9989	1.905	3.181	0.9978
21	2.810	3.714	^{a}	2.460	3.642	0.9950
22	1.965	3.755	0.9978	1.485	2.650	0.9970
23	1.996	3.229	0.9990	1.721	2.973	0.9978
24	3.023	4.024	^{a}	2.402	3.567	0.9937
25	1.739	2.983	0.9992	1.432	2.635	0.9976
26	1.289	2.508	0.9987	0.856	2.234	0.9984
27	1.075	2.178	0.9974	1.104	2.226	0.9990
28	1.260	2.418	0.9963	1.076	2.421	0.9950
29	1.881	3.042	0.9980	1.533	2.934	0.9972
30	1.899	3.128	0.9978	1.491	2.946	0.9981
31	3.441	4.758	^{a}	2.823	4.559	0.9923
32	2.410	3.804	0.9949	1.806	3.001	0.9957
33	1.971	2.448	0.9887	2.058	3.170	0.9979
34	–	–	–	2.588	3.910	0.9955
35	1.021	2.801	0.9995	0.554	2.471	0.9903
36	1.167	2.816	0.9994	0.664	2.317	0.9996
37	1.109	2.798	0.9987	0.614	2.462	0.9946
38	0.886	2.957	0.9978	0.621	2.147	0.9886
39	1.700	3.409	0.9994	0.933	2.455	0.9959
40	1.474	3.046	0.9986	1.175	2.686	0.9955
41	2.382	4.576	0.9996	1.446	3.206	0.9947
42	1.601	3.577	0.9982	0.894	2.573	0.9941
43	1.429	3.649	0.9978	0.816	2.847	0.9970
44	1.052	3.252	0.9985	0.474	1.958	0.9993
45	2.436	3.987	0.9965	1.456	2.819	0.9967
46	2.876	4.228	0.9986	1.969	3.422	0.9961

^{a} The constants a and m were determined using only two data points of capacity factors (for only two different mobile phase compositions), as in other mobile phases the solute is either too strongly or too weakly retained.

Note that the constants a_1 and p in water–methanol mobile phases do not depend significantly on the type of homologous series, in contrast to acetonitrile–water mobile phases. This is in agreement with the earlier results for other homologous series [14]. In acetonitrile–water mobile phases, alkylbenzenes and alkyluracils have values of a_1 and p approximately 2–3 times lower than the corresponding constants for alkoxycarbonylpyrazolines and alkanones, which are however close to each other.

The values of a_0 decrease with decreasing overall retention in a given homologous series, *i.e.* with increasing polarity and decreasing size of the end group (structural residue in the series). The index q increases in the same direction in methanol–water mobile phases, whereas no straightforward structural dependence of this constant is apparent in acetonitrile–water mobile phases.

TABLE III

CONSTANTS a_0, a_1, p AND q OF EQN. 2 FOR THE HOMOLOGOUS SERIES OF n-ALKYLBEN-ZENES (A), 3-n-ALKYL-6-METHYLURACILS (B), 3-ALKOXYCARBONYL-2-PYRAZOLINES (C) AND ALKAN-2-ONES (D) IN METHANOL–WATER (I) AND ACETONITRILE–WATER (II) MOBILE PHASES

Homologous series	Mobile phase	a_0	a_1	p	q
A	I	1.981	0.563	0.878	0.705
	II	2.296	0.282	0.401	2.718
B	I	− 0.300	0.560	0.771	2.116
	II	− 0.447	0.342	0.483	2.361
C	I	− 0.174	0.616	0.840	1.849
	II	− 0.639	0.545	1.109	1.375
D	I	− 0.561	0.605	0.828	1.372
	II	− 0.527	0.510	1.107	0.992

TABLE IV

INDICES Δn_c AND Δq OF SAMPLE SOLUTES RELATED TO TOLUENE (SOLUTE NO. 1) AS THE REFERENCE STANDARD (EQN. 5a AND b) IN METHANOL–WATER (I) AND ACETONITRILE–WATER (II) MOBILE PHASES, USING n-ALKYLBENZENES (A), 3-n-ALKYL-6-METHYLURACILS (B), 3-ALKOXYCARBONYL-2-PYRAZOLINES (C) AND ALKAN-2-ONES (D) AS THE CALIBRATION HOMOLOGOUS SERIES

Numbers of solutes are as in Table I.

Solute	Mobile phase	A		B		C		D	
		Δn_c	Δq	Δn_c	Δq	Δn_c	Δq	Δn_c	Δq
17	I	− 1.35	0.77	− 1.35	0.69	− 1.23	0.74	− 1.25	0.73
	II	− 3.29	− 0.47	− 2.72	− 0.39	− 1.71	0.19	− 1.82	0.18
18	I	− 0.62	0.32	− 0.62	0.29	− 0.57	0.31	− 0.58	0.31
	II	− 1.75	− 0.24	− 1.45	− 0.20	− 0.91	0.11	− 0.97	0.11
19	I	− 0.96	0.82	− 0.97	0.77	− 0.88	0.80	− 0.90	0.80
	II	− 3,78	− 0.62	− 3.12	− 0.53	− 1.96	0.14	− 2.09	0.13
20	I	− 1.12	0.48	− 1.12	0.42	− 1.02	0.46	− 1.04	0.45
	II	− 2.32	− 0.30	− 1.91	− 0.25	− 1.20	0.16	− 1.28	0.15
21	I	0.47	0.57	0.47	0.59	0.43	0.58	0.44	0.58
	II	− 0.35	− 0.06	− 0.29	− 0.06	− 0.18	0.00	− 0.19	0.00
22	I	− 1.03	0.75	− 1.04	0.69	− 0.95	0.73	− 0.96	0.72
	II	− 3.80	− 0.67	− 3.14	− 0.58	− 1.97	0.09	− 2.10	0.09
23	I	− 0.98	0.80	− 0.98	0.74	− 0.90	0.78	− 0.91	0.77
	II	− 2.97	0.44	− 2.45	− 0.37	− 1.54	0.15	− 1.64	0.15
24	I	0.84	0.69	0.85	0.74	0.77	0.71	0.79	0.71
	II	− 0.55	− 0.12	− 0.46	− 0.10	− 0.29	− 0.01	− 0.31	− 0.01
25	I	− 1.43	0.78	− 1.44	0.69	− 1.31	0.74	− 1.34	0.73
	II	− 3.99	− 0.66	− 3.30	− 0.57	− 2.07	0.14	− 2.21	0.13
26	I	− 2.24	0.70	− 2.25	0.56	− 2.04	0.65	− 2.08	0.63
	II	− 6.04	− 0.83	− 4.98	− 0.69	− 3.13	0.37	− 3.34	0.37
27	I	− 2.62	0.55	− 2.63	0.40	− 2.39	0.45	− 2.44	0.48
	II	− 5.48	− 0.90	− 4.52	− 0.77	− 2.84	0.19	− 3.23	0.18

TABLE IV (*continued*)

Solute	Mobile phase	A		B		C		D	
		Δn_c	Δq	Δn_c	Δq	Δn_c	Δq	Δn_c	Δq
28	I	− 2.28	0.63	− 2.30	0.49	− 2.09	0.58	− 2.13	0.57
	II	− 5.26	0.73	− 4.34	− 0.61	− 2.72	0.32	− 2.91	0.31
29	I	− 1.15	0.78	− 1.16	0.71	− 1.08	0.68	− 1.10	0.68
	II	− 3.63	− 0.40	− 3.00	− 0.32	− 1.88	0.32	− 2.01	0.32
30	I	− 1.18	0.71	− 1.19	0.64	− 1.05	0.76	− 1.07	0.75
	II	− 3.78	− 0.37	− 3.12	− 0.28	− 1.96	0.38	− 2.09	0.38
31	I	1.59	1.06	1.59	1.15	1.45	1.09	1.48	1.10
	II	0.94	0.71	0.77	0.68	0.48	0.52	0.52	0.51
32	I	− 0.24	1.01	− 0.25	0.99	− 0.22	1.00	− 0.23	1.00
	II	− 2.67	− 0.44	− 2.20	− 0.38	− 1.38	0.09	− 1.48	0.08
33	I	− 1.02	0.04	− 1.03	− 0.02	− 0.94	0.01	− 0.95	0.01
	II	− 1.77	− 0.38	− 1.47	− 0.33	− 0.92	− 0.02	− 0.98	− 0.03
34	I	0.48	0.56	0.48	0.59	0.44	0.57	0.45	0.58
	II	0.11	0.15	0.08	0.15	0.05	0.13	0.06	0.13
35	I	− 2.71	1.22	− 2.73	1.06	− 2.48	1.17	− 2.53	1.15
	II	− 7.11	− 0.47	− 5.86	− 0.31	− 3.68	0.95	− 3.93	0.94
36	I	− 2.45	1.11	− 2.46	0.96	− 2.24	1.06	− 2.28	1.04
	II	− 6.72	− 0.67	− 5.54	− 0.51	− 3.48	0.67	− 3.71	0.66
37	I	− 2.55	1.14	− 2.57	0.99	− 2.34	1.09	− 2.38	1.07
	II	− 6.89	− 0.50	− 5.69	− 0.34	− 3.57	0.87	− 3.81	0.86
38	I	− 2.95	1.50	− 2.97	1.32	− 2.70	1.44	− 2.75	1.42
	II	− 6.87	− 0.82	− 5.67	− 0.66	− 3.56	0.55	− 3.80	0.54
39	I	− 1.50	1.24	− 1.51	1.15	− 1.38	1.20	− 1.40	1.19
	II	− 5.76	− 0.64	− 4.75	− 0.51	− 2.99	0.51	− 3.19	0.50
40	I	− 1.90	1.07	− 1.92	0.96	− 1.74	1.03	− 1.78	1.02
	II	− 4.90	− 0.51	− 4.05	− 0.39	− 2.54	0.47	− 2.71	0.47
41	I	− 0.29	1.80	− 0.30	1.79	− 0.27	1.80	− 0.27	1.80
	II	− 3.94	− 0.09	− 3.25	0.00	− 2.04	0.69	− 2.18	0.69
42	I	− 1.68	1.49	− 1.69	1.39	− 1.54	1.45	− 1.57	1.44
	II	− 5.90	− 0.51	− 4.87	− 0.37	− 3.06	0.67	− 3.26	0.66
43	I	− 1.98	1.71	− 2.00	1.59	− 1.82	1.67	− 1.85	1.66
	II	− 6.18	− 0.20	− 5.10	− 0.06	− 3.20	1.03	− 3.42	1.02
44	I	− 2.65	1.65	− 2.67	1.47	− 2.43	1.59	− 2.47	1.57
	II	− 7.39	− 0.95	− 6.10	− 0.78	− 3.83	0.52	− 4.09	0.51
45	I	− 0.19	1.17	− 0.20	1.16	− 0.18	1.16	− 0.18	1.16
	II	− 3.91	− 0.48	− 3.23	− 0.39	− 2.03	0.29	− 2.16	0.29
46	I	0.59	1.02	0.59	1.06	0.53	1.04	0.54	1.04
	II	− 2.09	− 0.09	− 1.73	− 0.04	− 1.08	0.33	− 1.16	0.32

The differential structural indices Δn_c and Δq (eqn. 5a and b) of 30 test solutes, related to toluene as the reference standard and calculated using the constants a_0, a_1 and p of the four potential calibration series, are compared in Table IV. In water–methanol mobile phases, good agreement is observed between the differential indices Δn_c and Δq determined using all the individual homologous calibration series. The maximum difference between the individual Δn_c values for a given solute is 0.27, and only for seven compounds are these differences higher than 0.20. Only three differences between the indices Δq exceed 0.15, with the maximum value 0.18. For most

TABLE V

EXPERIMENTAL (E) AND PREDICTED (P) CAPACITY FACTORS k' OF SAMPLE SOLUTES IN METHANOL–WATER (I, 40 AND 25% METHANOL) AND IN ACETONITRILE–WATER (II, 40 and 30% ACETONITRILE) MOBILE PHASES

The prediction is based on calculations using eqns. 3–5 and the indices Δn_c and Δq listed in Table IV, with toluene as the reference standard and n-alkylbenzenes (A), 3-n-alkyl-6-methyluracils (B), 3-alkoxycarbonyl-2-pyrazolines (C) and alkan-2-ones (D) as the calibration homologous series. Numbers of solutes are as in Table I.

Solute	Mobile phase	k'				
		E	P(A)	P(B)	P(C)	P(D)
17	I, 40	3.83	3.62	3.64	3.64	3.65
	I, 25	10.13	9.89	9.60	9.94	9.97
	II, 40	2.81	2.79	2.76	2.76	2.80
	II, 30	5.98	6.29	6.25	6.24	6.31
18	I, 40	10.31	10.13	10.09	10.07	10.03
	I, 25	27.90	26.83	26.81	26.68	26.62
	II, 40	5.24	5.22	5.20	5.20	5.20
	II, 30	12.30	12.94	12.88	12.88	12.90
19	I, 40	4.82	4.80	4.75	4.79	4.74
	I, 25	14.86	14,26	14.13	14.22	14.10
	II, 40	2.48	2.45	2.44	2.43	2.46
	II, 30	4.91	5.28	5.26	5.24	5.28
20	I, 40	6.21	5.74	5.73	5.74	5.74
	I, 25	16.02	14.75	14.78	14.78	14.78
	II, 40	4.04	4.04	4.06	4.06	4.10
	II, 30	9.02	9.74	9.78	9.76	9.84
21	I, 40	21,12	20,12	20,23	20,12	20,23
	II, 40	9.12	9.49	9.55	9.58	9.59
	II, 30	24,62	25,42	25,54	25,62	25,66
22	I, 40	4.87	4.83	4.80	4.78	4.83
	I, 25	15.26	13.83	13.75	13.68	13.82
	II, 40	2.52	2.54	2.52	2.53	2.53
	II, 30	5.07	5.40	5.37	5.38	5.39
23	I, 40	5.22	4.81	4.84	4.78	4.83
	I, 25	15.35	14.14	14.22	14.06	14.19
	II, 40	3.24	3.23	3.22	3.23	3.24
	II, 30	6.98	7.40	7.39	7.40	7.42
24	I, 40	25.89	24.59	24.73	24.60	24.87
	II, 40	8.41	8.99	8.89	8.96	8.95
	II, 30	22.61	23.64	23.41	23.53	23.52
25	I, 40	3.60	3.36	3.36	3.37	3.36
	I, 25	9.65	9.07	9.07	9.09	9.03
	II, 40	2.26	2.27	2.26	2.25	2.27
	II, 30	4.43	4.81	4.79	4.78	4.81
26	I, 40	1.88	1.83	1.84	1.84	1.85
	I, 25	4.58	4.18	4.21	4.22	4.22
	II, 40	0.92	0.87	0.87	0.87	0.87
	II, 30	1.57	1.68	1.68	1.68	1.68
27	I, 40	1.52	1.52	1.52	1.52	1.52
	I, 25	3.44	3.11	3.10	3.11	3.09
	II, 40	1.33	1.26	1.25	1.26	1.27
	II, 30	2.26	2.42	2.42	2.42	2.44

TABLE V (*continued*)

Solute	Mobile phase	k'				
		E	P(A)	P(B)	P(C)	P(D)
28	I, 40	1.95	1.87	1.88	1.88	1.86
	II, 25	4.46	4.15	4.16	4.16	4.14
	II, 40	1.36	1.21	1.21	1.21	1.22
	II, 30	2.25	2.44	2.44	2.45	2.45
29	I, 40	4.67	4.24	4.23	4.43	4.40
	I, 25	12.61	12.04	12.00	12.17	12.11
	II, 40	2.16	2.17	2.17	2.18	2.17
	II, 30	4.67	4.94	4.93	4.95	4.94
30	I, 40	4.54	4.42	4.40	4.23	4.24
	I, 25	12.41	12.16	12.11	12.02	12.03
	II, 40	2.02	1.94	1.94	1.95	1.95
	II, 30	4.21	4.44	4.42	4.44	4.45
31	I, 40	34.46	32.87	32.79	32.89	33.03
	II, 40	8.83	9.43	9.47	9.41	9.55
	II, 30	31.81	31.20	31.27	31.09	31.49
32	I, 40	7.34	7.39	7.36	7.41	7.36
	I, 25	26.40	26.49	26.33	26.54	26.34
	II, 40	3.79	3.80	3.81	3.82	3.83
	II, 30	8.49	8.78	8.80	8.81	8.83
33	I, 40	9.75	9.36	9.31	9.36	9.37
	I, 25	20.71	21.03	20.91	20.94	21.02
	II, 40	5.92	5.87	5.79	5.82	5.88
	II, 30	13.31	14.09	13.90	13.98	14.10
34	I, 40	21.42	20.48	20.41	20.51	20.42
	II, 40	9.89	10.05	9.96	9.98	10.02
	II, 30	28.03	28.59	28.34	28.39	28.54
35	I, 40	0.77	0.76	0.76	0.75	0.75
	I, 25	2.13	1.93	1.91	1.91	1.90
	II, 40	0.34	0.35	0.35	0.35	0.35
	II, 30	0.69	0.71	0.71	0.71	0.71
36	I, 40	1.08	1.05	1.06	1.05	1.05
	I, 25	2.97	2.67	2.68	2.67	2.68
	II, 40	0.55	0.52	0.51	0.52	0.52
	II, 30	0.92	1.02	1.02	1.02	1.03
37	I, 40	0.94	0.94	0.93	0.93	0.93
	I, 25	2.57	2.37	2.35	2.34	2.35
	II, 40	0.41	0.40	0.40	0.40	0.41
	II, 30	0.79	0.82	0.82	0.82	0.83
38	I, 40	0.52	0.48	0.48	0.48	0.48
	I, 25	1.41	1.29	1.28	1.28	1.28
	II, 40	0.56	0.55	0.54	0.54	0.55
	II, 30	1.02	1.04	1.03	1.03	1.04
39	I, 40	2.18	2.07	2.07	2.07	2.08
	I, 25	7.15	6.48	6.47	6.45	6.48
	II, 40	0.86	0.85	0.85	0.84	0.85
	II, 30	1.64	1.73	1.73	1.71	1.73
40	I, 40	1.78	1.73	1.71	1.72	1.71
	I, 25	5.05	4.77	4.71	4.75	4.70
	II, 40	1.29	1.20	1.19	1.20	1.20
	II, 30	2.44	2.58	2.55	2.57	2.57

TABLE V (*continued*)

Solute	Mobile phase	k'				
		E	P(A)	P(B)	P(C)	P(D)
41	I, 40	3.61	3.42	3.37	3.39	3.39
	I, 25	17.61	15.98	15.77	15.83	15.90
	II, 40	1.36	1.38	1.38	1.38	1.38
	II, 30	3.26	3.34	3.34	3.35	3.34
42	I, 40	1.46	1.41	1.41	1.41	1.41
	I, 25	5.12	4.68	4.67	4.67	4.65
	II, 40	0.70	0.70	0.69	0.69	0.70
	II, 30	1.41	1.46	1.45	1.45	1.46
43	I, 40	0.93	0.90	0.89	0.89	0.89
	I, 25	3.37	3.04	3.02	3.01	3.01
	II, 40	0.47	0.45	0.45	0.45	0.45
	II, 30	0.96	1.00	1.00	1.00	1.01
44	I, 40	0.58	0.54	0.54	0.54	0.54
	I, 25	1.77	1.60	1.59	1.59	1.60
	II, 40	0.49	0.46	0.46	0.46	0.47
	II, 30	0.78	0.84	0.84	0.84	0.85
45	I, 40	6.62	6.65	6.59	6.64	6.66
	I, 25	26.69	25.41	25.15	25.32	25.39
	II, 40	2.02	2.01	2.00	2.02	2.02
	II, 30	4.26	4.45	4.43	4.45	4.48
46	I, 40	15.08	14.71	14.61	14.48	14.54
	I, 30	39.91	43.42	43.15	42.78	42.91
	II, 40	3.76	3.78	3.76	3.77	3.79
	II, 30	9.31	9.60	9.55	9.60	9.61

compounds maximum deviations are observed with the indices determined using *n*-alkylbenzenes as the calibration series. This can be understood if we consider the impact of the strongly limited number of the experimental data on the precision of determination of the constants a_0, a_1 and p used for the calibration of the retention scale. Deviations from the mean values of Δn_c and Δq in the opposite direction were found for the indices based on alkyluracils as the calibration series, while the indices determined using alkoxycarbonylpyrazoline and alkanone calibration series were almost identical.

In acetonitrile–water mobile phases, the indices Δn_c and Δq determined with alkylbenzenes as the calibration homologous series differ significantly from those determined with the calibration series of alkyluracils and even more from the values determined on the basis of the two remaining calibration series. On the other hand, the values of Δn_c and Δq for alkoxycarbonylyrazolines and alkanones as the calibration series are close to each other, with only seven differences in Δn_c exceeding 0.20 and with maximum differences in Δq indices of 0.01. The deviations observed can be attributed to the lack of experimental data for precise determination of the constants of the calibration eqn. 4 with alkylbenzene calibration series and to slightly curved log k' versus x plots of alkyluracils.

Note that the least differences between the indices Δn_c determined in acetonitrile–water and in methanol–water mobile phases are obtained with alkoxycarbo-

nylpyrazolines and with alkanones as the calibration series. The differences between the n_{ce} indices are even less, < 0.5 for the great majority of the sample solutes tested. Toluene is one of the exceptions, which explains the better agreement observed between the n_{ce} than between the Δn_c indices. These results suggest that alkoxycarbonylpyrazolines and alkanones are better suited as the calibration homologous series than alkylbenzenes and alkyluracils in the tested range of mobile phase compositions.

As the final test of the prediction method, the capacity factors predicted using eqns. 4 and 5a and b with the differential indices Δn_c and Δq and the calibration constants a_0, a_1 and p, determined using the four tested calibration homologous series, were compared with the experimental k' (Table V). Only the results for two concentrations of methanol (40 and 25%) and acetonitrile (40 and 30%) are shown, even though other mobile phase compositions were also tested.

All the predicted k' differ from the experimental values by less than 10% (relative to k'_{exp}) and the values predicted using various calibration homologous series differ only insignificantly from each other, which means that all the series tested can in principle be used for the calibration of retention in reversed-phase systems with mobile phases containing 25–50% methanol or 30–50% acetonitrile in water. In mobile phases containing 50% organic solvent, the differences between the predicted and experimental values are even less than those for the mobile phases in Table V. However, these differences may be as high as 20% rel. for some test solutes in 25% aqueous acetonitrile as the mobile phase, because of the curvature of the log k' *versus* x plots in this range of relatively high water contents.

CONCLUSIONS

The application of the method for prediction of retention in reversed-phase systems based on the indices Δn_c and Δq and the n-alkylbenzene calibration homologous series failed in water-rich mobile phases when the parameters of the calibration eqn. 4 were determined in mobile phases containing more than 50% organic solvent, but proved feasible with a suitable calibration homologous series the retention of which could be measured in mobile phases containing 25–50% methanol or acetonitrile. In principle, it is possible to use homologous n-alkylbenzenes as the basis of calibration, with the calibration constants determined only from the retention data of toluene over the full composition range of mobile phases and two additional data for ethylbenzene, however the indices Δn_c and Δq determined in this way may be subject to significant errors.

For better accuracy and consistency, it is preferable to use another calibration homologous series which allows the experimental determination of the capacity factors of the first four or five members in the investigated range of composition of mobile phases. Although the four homologous series tested here provided similar results, with the predicted k' differing by 10% rel. or less from the experimental values in 25–50% aqueous methanol or in 30–50% aqueous acetonitrile, the homologous alkoxycarbonylpyrazolines or alkan-2-ones are to be preferred because of a better consistency of the indices n_{ce} and q_i (Δn_c and Δq) determined in methanol–water and in acetonitrile–water mobile phases.

This accuracy is similar to previous results achieved using the present method in mobile phases containing 50–90% organic solvent [11,12] and better than that report-

ed using the method based on the parameters of the concentration dependences of the structural increments to retention indices [7]. The advantage of the latter method is that fewer preliminary experimental data are required for its application. Apparently a common feature of all the methods of calibration and prediction of retention suggested so far is that a decreased number of necessary experiments should be traded for either a limited range of experimental conditions for which the method is applicable or a decreased accuracy of the predicted retention data.

Taking into account the better availability of the alkan-2-ones, this homologous series is recommended for calibration in water-rich mobile phases using the method based on the indices Δn_c and Δq, as a supplement to the n-alkylbenzene calibration series, and is suitable for mobile phases with 50% or less water.

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