Retention indices of phenols for internal standards in reversed-phase high-performance liquid chromatography

Application to retention prediction and selectivities of mobile phases and packing materials

Shiro Yamauchi

Analytical Development, Formulation Research Institute, **Otsuka** *Pharmaceutical Co., Ltd.,* **224-18** *Ebisuno,* **Hiraishi**, *Kawauchi-cho, Tokushima 771-01 (Japan)*

ABSTRACT

The retention indices of previously reported phenols were calculated by means of the least-squares method on the basis of *n*-alkyl 4-hydroxybenzoate scales and applied to retention predictions and selectivities of mobile phases and packing materials. A simple method was established which predicts the retention times of phenols as candidates for internal standards in **reversed-phase** high-performance liquid chromatography. Relationships between log k' and the numbers of carbon atoms in the **alkyl** chain of *n*-alkyl 4-hydroxybenzoates, which are standard compounds for calculating retention indices, showed excellent linearity under all the tested conditions. The retention time of each phenol was calculated from the retention data for one marker solution consisting of uracil, methyl 4-hydroxybenzoate and ethyl Chydroxybenxoate and the retention index of each phenol. The experimentally observed retention times and predicted values agreed to **within** 10% relative error under several conditions. Using this technique, approximate retention times of phenols can be predicted easily and promptly. In addition, selectivities of the mobile phase and packing materials for phenols are discussed.

INTRODUCTION

Previously, we have reported a group of commercially available phenols and their utility **as** candidates for internal standards in RP-HPLC [1]. Their most valuable property is that they are eluted in a constant order regardless of the HPLC conditions that are used in pharmaceutical analysis with hydrocarbon chemically bonded silica gel columns. In the process of selecting an internal standard, the retention times of candidate compounds are usually determined and one compound is selected on the basis of the results. In a previous paper [1], we showed only their typical elution pattern in acetonitrile **(ACN)**– water **(20:80** to **60:40)** mobile phases with one particular **prepacked** ODS column (Nucleosil **5C$_{18}$,** 15 cm x 4.6 mm I.D.). However, the column size, flow-rate and temperature of the column oven and/or particle size of the packing material are not usually identical in each HPLC analysis even though the mobile phase and the kind of packing material are common. Therefore, the retention times of phenols in Fig. 1 in ref. 1 are not always useful.

We considered that a simple method for predicting retention times should be developed for the purpose of selecting a suitable internal standard among candidate phenols. Their elution order was estimated on the basis of the linearity of logarithmic plots of their capacity factors (k') under different conditions. Expressing each

phenol in terms of a suitable physico-chemical parameter (f), the retention time of each phenol will be able to be predicted by the following equation, based on a linear free energy relationship:

$$\log k' = af + b \tag{1}$$

where k' is the capacity factor of the analyte and a and b are constants.

There have been many investigations relevant to retention time predictions in liquid chromatography, and recently Skelly et al. [2] reported "computer-assisted internal standard selection (CAISS) in RP-HPLC". Based on investigations using eqn. 1, Smith and Burr [3–7] have attempted to predict retentions of many kinds of organic compounds by using retention indices based on alkyl aryl ketones and they have been successful in predicting retentions under a wide range of conditions. They used retention indices based on an alkyl aryl ketone scale as f and demonstrated their utility in retention predictions. On the other hand, Hanai and co-workers [8–14] have reported systems for retention predictions of many kinds of compounds by using the logarithms of partition coefficients in 1-octanol–water (log P) according to Rekker's fragment constants [15] as f. In this study, we chose retention index as f because the group of phenols that we selected include n-alkyl 4-hydroxybenzoates, which are suitable as standard compounds for calculating retention indices. In this paper, we report the method and some application results for retention prediction and, in addition, selectivities of the mobile phase and packing materials for phenols.

EXPERIMENTAL

n-Octyl 4-hydroxybenzoate was obtained from Lancaster Synthesis. Other chemicals, apparatus and procedures were as described previously [1]. There is no general standard compound or overall method for determining the column void volume, which is necessary for calculating capacity factors, and reviews of this issue have been published [16,17]. We chose uracil to determine the column void volume in this study. All the phenols used are listed in Table I.

TABLE I

PHENOLS USED IN THIS STUDY

The structure and name of each phenol are expressed using abbreviations of substituents: Me = CH_3-; Et = C_2H_5-; iso-Pr = $(CH_3)_2CH-$; *n*-Pr = $CH_3(CH_2)_2-$; *sec*.-Bu = $CH_3CH_2CH \cdot (CH_3)-$; *n*-Bu = $CH_3(CH_2)_3-$; iso-Am = $(CH_3)_2CH(CH_2)_2-$; n-Am = $CH_3(CH_2)_4-$;   n-Hex = $CH_3(CH_2)_5-$;   n-Hep = $CH_3(CH_2)_5-$; Oc = $CH_3(CH_2)_3CH(C_2H_5)CH_2-$; *n*-Oc = $CH_3 . (CH_2)_7-$; n-No = $CH_3(CH_2)_8-$; Ph = C_6H_5-.

No.	Derivative	Substituent
1	4-Hydroxyacetanilide	4-NHCOMe
2	3-Hydroxyacetanilide	3-NHCOMe
3	2-Hydroxyacetanilide	2-NHCOMe
4	4-Hydroxyacetophenone	4-COMe
5	3-Hydroxyacetophenone	3-COMe
6	Methyl 4-hydroxybenzoate	4-COOMe
7	4-Hydroxypropiophenone	4-COEt
8	3-Nitrophenol	3-NO,
9	Ethyl 4-hydroxybenzoate	4-COOEt
10	4-Chlorophenol	4-Cl
11	4-Bromophenol	4-Br
12	4-Hydroxybenzophenone	4-COPh
13	Isopropyl 4-hydroxybenzoate	4-COOiso-Pr
14	*n*-Propyl 4-hydroxybenzoate	4-COO*n*-Pr
15	4-Hydroxyvalerophenone	4-CO*n*-Bu
16	2,3-Dichlorophenol	2,3-Cl$_2$
17	2,5-Dichlorophenol	2,5-Cl$_2$
18	4-Isopropylphenol	4-iso-Pr
19	sec.-Butyl 4-hydroxybenzoate	4-COO*sec*.-Bu
20	4-Hydroxybiphenyl	4-Ph
21	*n*-Butyl 4-hydroxybenzoate	4-COO*n*-Bu
22	4-*tert*.-Butyphenol	4-*tert*.-Bu
23	Benzyl 4-hydroxybenzoate	4-COOCH$_2$Ph
24	Thymol	3-Me-6-iso-Pr
25	Isoamyl 4-hydroxybenzoate	4-COOiso-Am
26	*n*-Amyl 4-hydroxybenzoate	4-COO*n*-Am
27	*n*-Hexyl 4-hydroxybenzoate	4-COO*n*-Hex
28	*n*-Heptyl 4-hydroxybenzoate	4-COO*n*-Hep
29	2-Ethyl-hexyl 4-hydroxybenzoate	4-coo-oc
30	*n*-Octyl 4-hydroxybenzoate	4-COO*n*-Oc
31	*n*-Nonyl 4-hydroxybenzoate	4-COO*n*-No

RESULT AND DISCUSSION

Linear relationship between log k' and the carbon numbers of alkyl chain in alkyl 4-hydroxybenzoates

In RP-HPLC, there is a linear relationship between log k' and carbon numbers of homologues, e.g., 2-ketoalkanes [18], 1-nitroalkanes [19], alkyl aryl ketones [20] and 1-phenylalkanes

[21]. We plotted log k' against the carbon number of the alkyl chain in alkyl **4-hydroxybenzoates.** As can be seen in Figs. 1 and 2, the relationship between log *k'* and carbon number seems to be linear. However, the point for the compound with an alkyl carbon number of 1, *i.e.,* methyl Chydroxybenzoate, deviates from each line. These results are similar to what Smith and Burr [5] found with **alkylben-zenes** and **Bogusz** and Aderjan [19] with 1-nitroalkanes. In order to determine the extent of the deviation of methyl **4-hydroxybenzoate** from the log *k' vs.* number lines, we determined the values of α_n, which is a relative increment of log *k'* per methylene unit in alkyl **4-hydroxyben-**zoates, defined by the following equation:

$$\alpha_n = (\log k'_n - \log k'_{n-1})/(\log k'_{n+1} - \log k'_n) \quad (2)$$

where $n + 1$, n and n − 1 are the carbon numbers of the alkyl chain in alkyl Chydroxybenzoates. α_n was found to be 0.89 ± 0.01 for $n = 2$ and 0.98 ± 0.02 for n = 3-8. We found that the increment of log *k'* per methylene unit between methyl and ethyl is about 90% of the others. This result indicates that the carbon number of methyl 4-hydroxybenzoate should be estimated as 1.1. This is very significant in the determination of the constants a and *b* in eqn. 1, as described later. The regression results between log *k'* and carbon number are shown in Table II, where the carbon number of methyl **4-hydroxy-**benzoate is 1.1 in accordance with the above result. Excellent linearity was shown under all conditions.

Calculation of retention indices

Based on alkyl 4-hydroxybenzoate scales, the retention index of each phenol was calculated by the least-squares method as reported by Smith and Burr [3,20]. In this process the retention indices of alkyl **4-hydroxybenzoates** are equal to 100 times the carbon number of the alkyl chain in alkyl Chydroxybenzoates except for methyl Chydroxybenzoate, whose retention index is 110. The results of the calculations are given in Tables III-V. None of the values depend on **pH (2.0–7.0),** ionic strength or counter ion (alkyl **sulphonates)** , as reported previously [1].
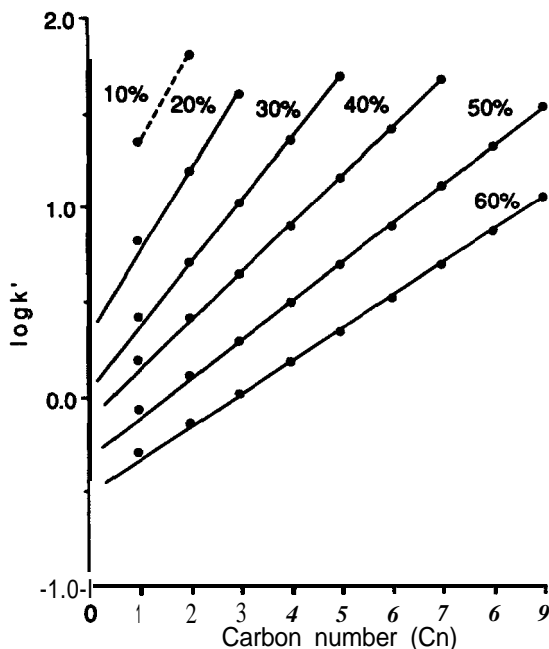

Fig. 1. Relationship between log k' and carbon number in acetonitrile-water mobile phases with Nucleosil 5C$_{18}$ column. Percentage values on lines indicate acetonitrile concentration.
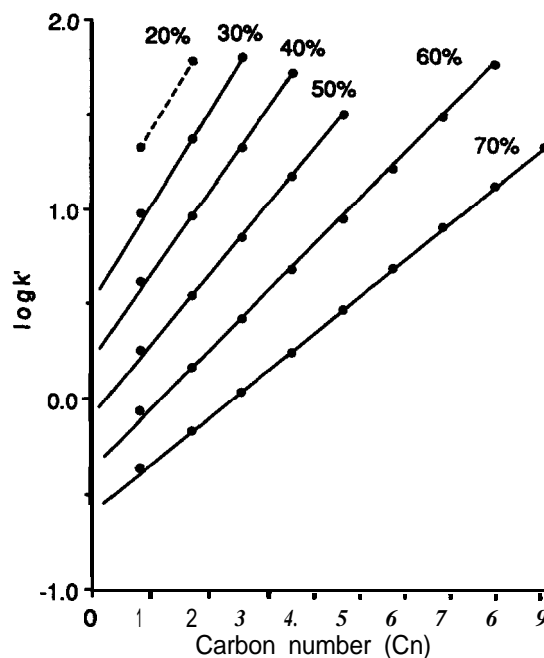

Fig. 2. Relationship between log *k'* and carbon number in methanol-water mobile phases with Nucleosil 5C$_{18}$ column. Percentage values on lines indicate methanol concentrations.

TABLE II

LINEAR RELATIONSHIP BETWEEN LOG k' OF n-ALKYL **4-HYDROXYBENZOATES** AND CARBON NUMBER IN RP-HPLC

Log $k' = a \cdot 100C_n + b.C_n$ is carbon number of alkyl chain in **n-alkyl 4-hydroxybenzoates,** except for methyl **4-hydroxybenzoate**, C_n for methyl **4-hydroxybenzoate** is 1.1.

Column	Mobile phase	a ($\times 1000$)	b	r [a]	n [b] (range of alkyl group used in the correlation)
Nucleosil SC,,	ACN-water **(10:90)**	5.078	0.793	–	2 (methyl to ethyl)
	ACN-water (2080)	4.111	0.374	0.99999	3 (methyl to propyl)
	ACN-water **(30:70)**	3.257	0.060	0.9999	4 (methyl to butyl)
	ACN-water **(40:60)**	2.521	-0.093	0.9998	7 (methyl to heptyl)
	ACN-water **(50:50)**	2.030	-0.301	0.9997	9 (methyl to nonyl)
	ACN-water **(60:40)**	1.704	-0.487	0.9997	9 (methyl to nonyl)
	MeOH–water (20:80)	5.011	0.781	–	2 (methyl to ethyl)
	MeOH–water (30:70)	4.373	0.500	0.99998	3 (methyl to propyl)
	MeOH–water (40:60)	3.776	0.207	0.99995	4 (methyl to butyl)
	MeOH–water (50:50)	3.198	-0.096	0.9999	5 (methyl to amyl)
	MeOH–water (60:40)	2.656	-0.363	0.9998	8 (methyl to **octyl)**
	MeOH–water (70:30)	2.158	-0.599	0.99997	9 (methyl to nonyl)
Chemcosorb **5-ODS-H**	ACN-water (30:70)	3.466	0.019	0.99994	5 (methyl to amyl)
YMC Pack ODS-A, A-302	ACN-water (30:70)	3.521	0.008	0.9999	5 (methyl to amyl)
Nucleosil 5C$_{18}$	ACN-water (30:70)	3.257	0.060	0.99993	5 (methyl to amyl)
µBondapak C$_{18}$	ACN-water (30:70)	2.983	-0.219	0.9998	5 (methyl to amyl)
Zorbax ODS	ACN-water (30:70)	3.357	-0.024	0.9999	5 (methyl to amyl)
Cosmosil 5Ph	ACN-water (30:70)	2.487	-0.004	0.999995	5 (methyl to amyl)
Nucleosil 5C$_8$	ACN-water (30:70)	2.763	0.025	0.999994	6 (methyl to hexyl)
Nucleosil 5C$_{18}$	ACN-water (30:70)	3.257	0.060	0.99993	5 (methyl to amyl)
	MeOH–water (4060)	3.776	0.207	099995	4 (methyl to butyl
	ACN-THF-water **(25:5:70)**	3.353	0.140	0.99996	4 (methyl to butyl)
	ACN-MeOH-water **(20:20:60)**	3.299	-0.005	0.9999	5 (methyl to amyl)
	ACN-MeOH-THF-water **(1:1:1:7)**	3.501	0.271	0.9998	4 (methyl to butyl)

[a] r = Correlation coefficient.
[b] n = Number of data points.

Long-term reproducibility and universality

Reproducibility and universality of the calculated retention indices of phenols are very important [4]. These were checked over 2 years under various chromatographic conditions. The difference between the maximum and minimum value for each phenol was not more than 10 retention index units. Hence long-term reproducibility and universality were confirmed.

Influence of temperature

All phenols were chromatographed using a Nucleosil 5C$_{18}$ prepacked column with ACN–water (40:60) as eluent at 25, 35, 45 and 55°C.

The calculated retention indices of each phenol did not change with variation in the temperature of the column oven.

Selectivities of mobile phases and packing materials for phenols

As can be seen from Tables III and IV, the retention indices of phenols having an alkylcarbonate group (of course, n-alkyl carbonate groups are excluded) or an alkylcarbonyl group are independent of the content of organic solvents [ACN or methanol (MeOH)] and the kind of organic solvent in the mobile phase. On the other hand, those of phenols in which the sub-

TABLE III

RETENTION INDICES OF PHENOLS IN ACETONITRILE-WATER AND METHANOL-WATER MOBILE PHASE WITH NUCLEOSIL $5C_{18}$ COLUMN

Values of *n*-alkyl Chydroxybenxoates are 100 times the carbon number in the alkyl chain of ester groups except for methyl 4-hydroxybenxoate.

No.	Phenol[a]	Retention index											
		Acetonitrile (%)						Methanol (%)					
		10	20	30	40	50	60	20	30	40	50	60	70
1	4-NHCOMe	-118	-148	-154	—		—	-86	-155	—	—	—	—
2	3-NHCOMe	-62	-86	-97	-103		—	-74	-95	-103	—	—	—
3	2-NHCOMe	-40	-41	-30	-24	-20	-33	-55	-59	-54	-52	-54	-54
4	4-COMe	29	19	23	20	26	9	34	13	15	6	4	2
5	3-COMe	48	44	49	50	59	53	40	27	30	21	20	17
6	4-COOMe	110	110	110	110	110	110	110	110	110	110	110	110
7	4-COEt	125	119	124	122	131	123	119	110	111	107	110	113
8	3-NO₂	—	141	166	173	184	176	—	—	105	116	134	147
10	4-Cl	—	199	222	231	247	243	—	147	177	187	205	219
11	4-Br	—	230	252	258	272	274	—	179	208	218	238	254
12	4-COPh	—	290	281	269	269	257	—	275	274	259	256	255
13	4-COOiso-Pr	—	284	286	281	284	281	—	276	276	269	268	272
15	4-CO*n*-Bu	—	313	315	309	314	308	—	302	298	302	301	306
16	2,3-Cl₂	—		311	319	326	330	—	—	264	282	288	305
17	2,5-Cl₂	—	—	332	343	358	356	—	—	276	302	312	331
18	4-iso-Pr			335	342	354	359	—	—	286	297	310	323
19	4-COO*sec.*-Bu	—		373	367	371	365	—	—	361	359	352	353
20	4-Ph			391	387	383	380	—	—	352	360	357	367
22	4-*tert.*-Bu			398	403	412	416	—	—	352	362	373	387
23	4-COOCH₂Ph			431	412	403	382	—	—	417	409	3%	393
24	3-Me-6-iso-Pr	—	—	437	456	468	476	—	—	375	397	406	424
25	4-COOiso-Am	—	—	488	479	477	473	—	—	—	477	476	478
29	4-coo-oc			—	—	750	749	—	—	—		745	745

[a] Each phenol is expressed *using* abbreviations of substituents (see Table I).

stituent group is nitro, halogen or alkyl do show a slight dependence. The retention indices increase with increasing the content of organic solvent, indicating that these phenols are more retained than alkylcarbonylphenols and alkyl 4-hydroxybenzoates in a more hydrophobic mobile phase.

The influence of the kind of organic solvent in the mobile phase on selectivity was evaluated from Table IV The eluotropic strengths of mobile phases I [ACN-water (30:70)] and II (MeOH–water (40:60)] are almost equal in RP-HPLC [22,23]. It seems that phenols in which the substituent group is nitro, halogen or alkyl are more retained in ACN than MeOH by comparison with phenols having carbonyl groups (–NHCOMe, -COR or –COOR, R = alkyl).

Further, in mixtures of organic solvents, additive properties of the selectivity were recognized qualitatively. The selectivity of the packing materials for phenols can be evaluated from Table V. As expected, the three phenols having substituents that include a phenyl group (Nos. 12, 20 and 23) are more retained with a phenyl column (column F in Table V) owing to $\pi-\pi$ interactions. The selectivities of packing materials for other phenols are almost equal with all columns, C_{18}, C_8 and phenyl. As indicated later under

TABLE IV

RETENTION INDICES OF PHENOLS IN VARIOUS MOBILE PHASES WITH NUCLEOSIL 5C$_{18}$ COLUMN

Values of n-alkyl 4-hydroxybenzoates are 100 times the carbon number in the alkyl chain of ester groups except for methyl 4-hydroxybenxoate.

No.	Phenol"	Mobile phase*				
		I	II	III	IV	V
1	4-NHCOMe	− 154	-169	− 179	-164	-183
2	3-NHCOMe	- 9 7	− 103	-109	- 9 7	− 104
3	2-NHCOMe	- 3 0	- 5 4	- 5 0	- 3 2	- 5 4
4	4-COMe	23	15	11	15	1
5	3-COMe	49	30	37	42	26
6	4-COOMe	110	110	110	110	110
7	4-COEt	124	111	114	120	110
8	3-NO$_2$	166	105	174	148	187
10	4-Cl	222	177	237	204	253
11	4-Br	252	208	268	236	285
12	4-COPh	281	274	278	276	277
13	4-COOiso-Pr	286	276	286	277	281
15	4-CO*n*-Bu	315	298	312	311	310
16	2,3-Cl$_2$	311	264	328	294	353
17	2,5-Cl$_2$	332	276	350	309	372
18	4-iso-Pr	335	286	337	313	335
19	4-COO*sec.*-Bu	373	361	373	364	372
20	4-Ph	391	352	393	369	398
22	4-*tert.*-Bu	398	352	402	378	402
23	4-COOCH$_2$Ph	431	417	431	418	438
24	3-Me-6-iso-Pr	437	375	441	411	442
25	4-COOiso-Am	488	_	490	484	_
29	4-COO-Oc	_	_	_	_	_

^{a} Each phenol is expressed using abbreviations of substituents (see Table I).
^{b} I = ACN-water (30:70); II = MeOH–water (40:60); III = ACN-THF-water (25:5:70); IV= ACN-MeOH-water (20:20:60);
V= ACN-MeOH-THF-water (10:10:10:70).

Application, with the C$_4$ column the selectivity was also the same as for the others.

A phenolic **hydroxy** group hardly seems to interact with residual silanol groups, as in Table V the retention indices of phenols, except for n-alkyl **4-hydroxybenzoates,** with column E, which is not end-capped, are almost identical with those with end-capped ODS columns (columns A-D), and the dissociation constants (pK_a) of phenols listed in Table I are within the range 7.5-10.2. If a phenolic hydroxy group interacts with residual silanol groups under the conditions of this study, the difference in retention indices between non-end-capped and **end**-capped columns should be recognized in accordance with the pK_a values.

Determination of the coefficients a and b

For calculating the retention times of the phenols from eqn. 1, first the regression co-efficients **a** and **b** must be determined. Our method is different from Smith and Burr's [7] or Hanai and Hubert's [8] methods. In our proce-dure, a marker solution consisting of uracil, methyl **4-hydroxybenzoate** (MHB) and ethyl 4-hydroxybenzoate (EHB) is chromatographed under any HPLC conditions. The sample solvent is methanol in each instance because the peak shapes and retention times of three components were independent of the sample solvents [24,25], which were water, methanol-water (50:50), methanol and acetonitrile, under the HPLC conditions used in this study. As MHB and EHB

TABLE V

RETENTION INDICES OF PHENOLS IN ACETONITRILE-WATER (30:70) WITH VARIOUS COMMERCIALLY AVAILABLE COLUMNS

Values of n-alkyl 4-hydroxybenzoates are 100 times the carbon number in the alkyl chain of ester groups except for methyl 4-hydroxybenwate.

No.	Phenol"	Column*						
		A	B	C	D	E	F	G
1	4-NHCOMe	− 148	− 148	-154	− 138	-156	− 197	-176
2	3-NHCOMe	-92	-92	-97	-87	− 102	-123	-114
3	2-NHCOMe	-28	-27	-30	-26	− 24	-51	-45
4	4-COMe	22	23	23	24	19	16	15
5	3-COMe	52	54	49	48	46	45	39
6	4-COOMe	110	110	110	110	110	110	110
7	4-COEt	123	123	124	122	121	120	119
8	3-NO$_2$	167	170	166	161	158	177	165
10	4-Cl	225	229	222	220	227	234	214
11	4-Br	257	259	252	248	254	260	246
12	4-COPh	274	273	281	280	273	307	2%
13	4-COOiso-Pr	284	282	286	281	279	287	287
15	4-CO*n*-Bu	314	314	315	309	312	317	309
16	2,3-Cl$_2$	314	315	311	299	315	328	311
17	2,5-Cl$_2$	333	333	332	319	333	343	328
18	4-iso-Pr	340	340	335	329	339	341	322
19	4-COO*sec.*-Bu	372	372	373	366	369	376	373
20	4-Ph	390	390	391	377	390	429	3%
22	4-*tert.*-Bu	404	403	398	394	402	404	384
23	4-COOCH$_2$Ph	425	423	431	421	423	476	441
24	3-Me-6-iso-Pr	444	445	437	422	443	452	427
25	4-COOiso-Am	486	483	488	485	483	483	483
29	4-coo-oc	–	–		–		–	

[a] Each phenol is expressed using abbreviations of substituents (see Table I).
[b] A = Chemcosorb 5-ODS-H; B = YMC Pack ODS-A, A-302; C = Nucleosil 5C$_{18}$; D = μ Bondapak C$_{18}$; E = Zorbax ODS; F = Cosmosil 5Ph; G = Nucleosil 5C$_8$.

are the first two n-alkyl Chydroxybenzoate homologues and they are eluted faster than other homologues, the time required to **chromato**graph the marker solution can be reduced. The excellent linear relationships between log k' and the carbon number of the alkyl chain in alkyl **4-hydroxybenzoates** (Table II) make it possible to determine accurately coefficients **a** and b in eqn. 1 from only the results for one marker solution. As the f values are 110 and 200 for MHB and EHB, respectively, in eqn. 1, **a** and **b** can be calculated using the following equations:

$$a = \frac{1}{90} (\log k'_{Et} - \log k'_{Me}) \quad (3)$$

$$b = \frac{1}{9} (20 \log k'_{Me} - 11 \log k'_{Et}) \quad (4)$$

where k'_{Me} and k'_{Et} are the capacity factors of MHB and EHB, respectively. We adopted this procedure for determining **a** and **b** because these coefficients change with not only the kind and percentage of organic solvents in the mobile phase and the kind of column but also the manufacturer of columns with nominally the same packing material, e.g., octadecylsilica gels. Hence we think that the best method is determination from experimental data. In this procedure, from experimental retention data for only

one marker solution, we can determine the most important parameters (**a** and **b**) in predicting retention data simply, exactly and promptly.

Some retention indices were dependent on the chromatographic conditions. Smith and Burr [3-7] and West [26] reported a quadratic relationship and a linear relationship between retention indices and the percentage of miscible organic solvents in binary mobile phases, respectively, and were successful in predicting the retentions of a large number of organic compounds over a wide range of mobile phases in RP-HPLC. However, our aim is to predict the retention times of the 31 phenols using previously reported HPLC conditions, and the variation of the retention indices is not so large. Accordingly, we decided to use the average values in Tables III to IV to simplify the procedure. The average values are given in Table VI.

The procedure for predicting the retention times of phenols is as follows. In the first step, the marker solution has to be chromatographed. Over the range of previously reported conditions [1], the elution order of three compounds in the marker solutions is always uracil, MHB and EHB. From this result, **a** and **b** are calculated through eqns. 3 and 4 experimentally. Next, a, **b**, the retention indices in Table VI and the retention time (t_0) of uracil are substituted in the

following equation and finally the calculated retention times (t) of each phenol can be obtained:

$$t = t_0[1 + 10^{(aI+b)}] \tag{5}$$

In the chromatography of the marker solution, if after the first compound (uracil) has been eluted the second (MHB) is not eluted within a reasonable time in organic solvent-poor mobile phases, the probable candidates are limited to five kinds of phenols (Nos. l-5 in Table I).

APPLICATION

In order to demonstrate the utility of the proposed method for predicting the retention times of phenols, we applied it under several sets of conditions that we have been using for quantitative analysis by RP-HPLC. The predicted and observed values are shown in Fig. 3, and agree within a 10% relative error. Fig. 3C is a good comparison demonstrating the significance of why the retention index of methyl **4-hydroxyben**zoate was defined as 110. If 100 had been used, the relative error between the observed and predicted retention times of Nos. 25-31 would be 14–31%, whereas in Fig. 3C it is less than 4%.

TABLE VI

RETENTION INDICES (1) OF PHENOLS USED FOR CALCULATING RETENTION TIMES IN RP-HPLC

| No.[a]	Substituent"	I	No.[a]	Substituent"	I
1	4-NHCOMe	-148	17	2,5-Cl$_2$	331
2	3-NHCOMe	-93	18	4-iso-Pr	326
3	2-NHCOMe	-43	19	4-COO*sec.*-Bu	365
4	4-COMe	15	20	4-Ph	376
5	3-COMe	38	21	4-COO*n*-Bu	400
6	4-COOMe	110	22	4-*tert.*-Bu	390
7	4-COEt	117	23	4-COOCH$_2$Ph	412
8	3-NO$_2$	148	24	3-Me-6-iso-Pr	430
9	4-COOEt	200	25	4-COOiso-Am	480
10	4-Cl	213	26	4-COO*n*-Am	500
11	4-Br	244	27	4-COO*n*-Hex	600
12	4-COPh	270	28	4-COO*n*-Hep	700
13	4-COOiso-Pr	279	29	4-coo-oc	747
14	4-COO*n*-Pr	300	30	4-COO*n*-Oc	800
15	4-CO*n*-Bu	309	31	4-COO*n*-No	900
16	2,3-Cl$_2$	309			

[a] See Table I.
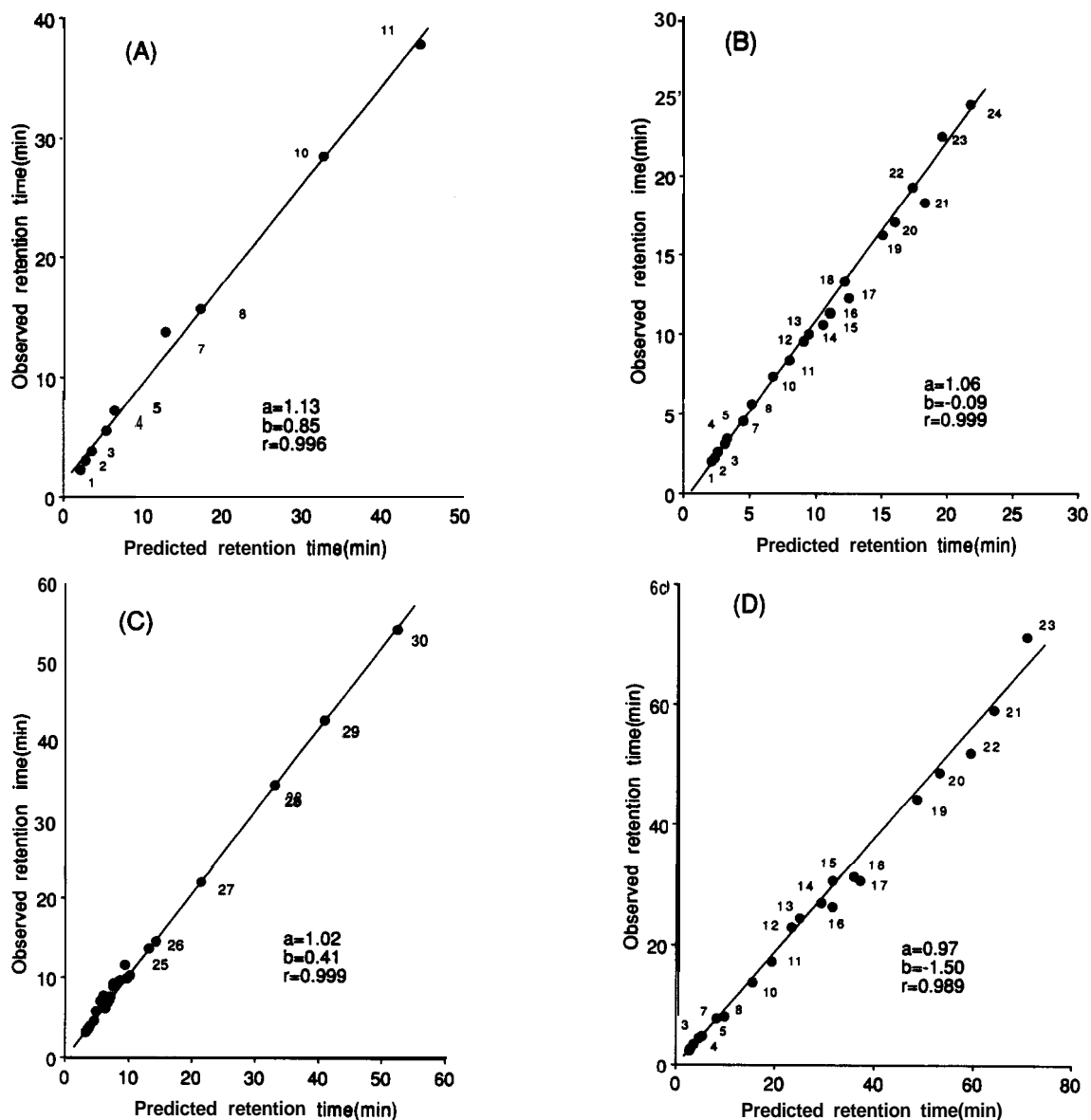
Fig. 3. Plots of predicted retention times using the proposed method and observed retention times under various conditions. *a = Slope; b* = intercept; r = correlation coefficient. Phenols are expressed using the numbers listed in Table I. Columns and mobile phases: (A) μBondasphere 5 μm C$_{18}$ and ACN-50 mM sodium sulphate-acetic acid (18:82:1); (B) μBondasphere 5 μm C$_4$ and ACN-water (30:70); (C) YMC Pack ODS-A, A-303 and ACN-MeOH-THF-20 mM sodium sulphate (5:5:5:12); (D) LiChrosorb RP-18 and MeOH–water (45:55).

CONCLUSION

For the purpose of assisting the selection of suitable internal standards among our candidate phenols in RP-HPLC, we established a simple method for predicting the retention times of 31 phenols. Excellent linear relationships between log **k'** and the carbon number of the alkyl chain in n-alkyl **4-hydroxybenzoates** were obtained for a wide range of HPLC conditions and retention indices were calculated on the basis of an n-alkyl **4-hydroxybenzoate** scale by the least-squares method. In addition, the selectivities of mobile phases and packing materials were discussed on

the basis of calculated retention index values. Constant retention index values, which are averages, were used in predicting retention times in order to simplify the procedure. The retention time of each phenol could be calculated from the retention data for one marker solution consisting of uracil, methyl 4-hydroxybenzoate and ethyl 4-hydroxybenzoate and the retention index of each phenol. The differences between predicted and observed retention times were not more than 10%. By using this method, we could obtain easily and promptly the approximate predicted retention times of 31 phenols from retention data for only one marker solution, without any trial-and-error experiments.

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