Correlations Between Retention Data of Isomeric Alkylbenzenes and Physical Parameters in Reversed-Phase Micro High-Performance Liquid Chromatography

K. Jinno* / K. Kawasaki

School of Materials Science, Toyohashi University of Technology, Toyohashi 440, Japan

Key Words

Micro-HPLC
Reversed Phase Chromatography
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Effect of chain length of bonded stationary phase

Summary

The separation of alkylbenzenes on reversed-phase C_2, C_8 and C_{18} columns by HPLC have been investigated. The correlation between log k' and physical parameters, such as the van der Waals volume, molecular connectivity index and hydrophobic parameter was studied.

Introduction

In liquid chromatography retention parameters such as the capacity factor are characteristic of the nature and molecular structure of the solutes and the stationary phases. Various authors have shown that correlations exist between the chromatographic retention data and a number of physical parameters such as the molecular connectivity index [1–4], shape parameter [5–8], hydrophobic parameter [9–16] and the van der Waals volume [17–20]. Over the past several years, much effort has been directed to investigate the retention mechanism in reversed-phase liquid chromatography (RPLC). During this period there have been major advances in the understanding of this mechanism. At present, it is generally considered that the retention of a solute in RPLC is due to a hydrophobic effect between the non-polar stationary phase, the polar mobile phase and the solute; this explanation is called as the solvophobic theory [21–26]. Thus, it is expected that some correlations exist between the chromatographic retention in RPLC and the hydrophobic parameter of a solute. Investigations in this field have been reported by a number of authors [9–16, 27–29].

Concerning now alkylbenzenes as solutes, the absence of polar functional groups in their molecules suggests that they could serve as useful models to test the solvophobic theory of retention which relates the retention parameter to the hydrophobic nature of the solute. According to literature data, while, for homologues n-alkylbenzenes, high correlation was found between the chromatographic retention and the physical parameters such as the hydrophobic parameter and/or the van der Waals volume [30–32], only relatively poor correlation was found for isomeric alkylbenzenes [30].

In the present work, a number of alkylbenzenes have been examined on three reversed-phase columns. The retention data have been related to the hydrophobic parameter, the van der Waals volume, the molecular connectivity index and their combination in order to establish the dominant parameter in controlling the retention of isomeric alkylbenzenes in RPLC.

Experimental

The liquid chromatographic system used consisted of the Model MF-2 microfeeder (Azuma Electric, Co., Ltd., Tokyo, Japan) used as the pump and the Uvidec 100 III ultraviolet detector (Jasco, Co. Ltd., Tokyo, Japan) set at 208nm.

The following three columns were prepared using the slurry technique:

a) a packed teflon C_{18} column:
teflon tubing of 0.5 mm i.d. × 12cm length was packed with Jasco FineSil C-18 (5μm).
b) a packed teflon C_2 column:
teflon tubing of 0.5 mm i.d. × 12cm length was packed with Jasco FineSil C-2 (10μm).
c) a packed teflon C_8 column:
teflon tubing of 0.5 mm i.d. × 12cm length was packed with Jasco FineSil C-8 (10μm).

The mobile phases consisted of HPLC-grade acetonitrile purchased from Kanto Chemicals (Tokyo, Japan) and purified water. All of the test substances (alkylbenzenes) were of the commercially available highest grades. Typical flow rate was 8μl/min.

Prior to the measurements the columns were washed with each mobile phase until a constant value was obtained for the retention of the test substance. The test solutes were analysed as solutions in acetonitrile, having a concentration of a few hundred parts per million. The retention time

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of sodium nitrite present at a concentration of about 100 ppm was taken as t_0 and the capacity factor, k′, was calculated from the retention time of the solute (t_R) according to the equation of $k' = (t_R - t_0)/t_0$. All measurements were made in triplicate. The average reproducibility of each run was better than 1.0% relative.

Results and Discussion

In the present work, the k′ values of 19 alkylbenzenes have been determined on the three reversed-phase stationary phases, the composition of the mobile phase being chosen to give reasonable retention times in each case. The results are listed in Table I, together with the van der Waals volume [33], molecular connectivity index [34] and hydrophobic parameter [35] values of each alkylbenzene.

Correlation with a single physical parameter

The correlation between the logarithm of the capacity factor (log k′) and the various physical parameters was investigated by linear regression analysis. The correlation coefficients obtained are listed in Table II for each data set. As reference, the table also lists the results reported by Smith [30]. The values in italics in Table II indicate us the parameter which gives the highest correlation coefficient. These results can be evaluated as follows:

(1) The van der Waals volume shows a reasonable correlation with log k′ obtained on C_2, C_8, C_{18} and C_{22} stationary phases.

(2) Correlation between log k′ and the connectivity index improves with decreasing alkyl chain length of the stationary phase.

(3) Correlation between log k′ and the van der Waals volume or the hydrophobic parameter increases with increasing alkyl chain length of the stationary phase.

These conclusions give us a clear overview of the retention mechanism in RPLC. The size and shape of a solute molecule, which are shown by the van der Waals volume and the connectivity index respectively, are the dominant factors controlling the retention of the solute on stationary phases with shorter alkyl-chains. In the case of longer alkyl chain the hydrophobic interaction is the dominant factor. In the case of the C_8 column, the effects of the three factors, i.e., molecular size, shape and hydrophobicity of a solute, equally contribute to the retention of alkylbenzenes in RPLC.

Investigation of the correlation between the capacity factor values measured on various phases also shows the existence of the above described concept of the retention mechanism, because a higher correlation implies a similarity of the interaction in the systems for a given solute type. The results of linear regression analysis of each data set are tabulated in Table III. High correlations are found between C_2 and C_8, as well as between C_{18} and C_{22}, while the lowest correlation was found between C_2 and C_{18}. This observation seems to be opposite to the results of our previous paper [36] where the interactions between the solute and the bonded stationary phases C_2, C_8 and C_{18} are almost similar in the mobile phase range investigated. However, the measurements of our previous paper only include three isomeric alkylbenzenes among the six test substances (toluene, ethylbenzene, 2-ethyl-1-methylbenzene, 1,3,5-trimethylbenzene, 1,2-diethylbenzene and n-butylbenzene). Similar evaluation of the present data set for the same three solutes gives almost the same results as obtained previously [36].

Table I. Capacity factor values and various physical parameters of alkylbenzenes on three reversed-phase columns

solute	capacity factor, k′			physical parameter		
	C_2 column	C_8 column	C_{18} column	log P	V_W	χ
Isopropylbenzene	1.47	2.14	1.48	3.65	79.96	3.352
1-Ethyl-2-methylbenzene	1.39	2.11	1.67	3.78	80.89	3.388
1-Ethyl-3-methylbenzene	1.51	2.24	1.51	3.78	80.89	3.382
1-Ethyl-4-methylbenzene	1.57	2.26	1.56	3.78	80.89	3.382
1,2,3-Trimethylbenzene	1.39	2.10	1.63	3.81	81.81	3.244
1,2,4-Trimethylbenzene	1.50	2.19	1.47	3.81	81.81	3.238
1,3,5-Trimethylbenzene	1.49	2.22	1.64	3.81	81.81	3.232
Isobutylbenzene	2.03	3.15	2.06	4.18	90.19	3.827
sec. Butylbenzene	1.86	2.86	1.84	4.18	90.19	3.892
tert. Butylbenzene	1.79	2.63	1.80	4.11	90.18	3.661
1-Isopropyl-4-methylbenzene	1.95	3.03	2.11	4.21	91.11	3.571
1,2-Diethylbenzene	1.81	2.92	1.97	4.31	91.12	3.949
1,3-Diethylbenzene	1.91	2.89	1.78	4.31	91.12	3.943
1,4-Diethylbenzene	1.94	2.91	1.90	4.31	91.12	3.943
1,2,3,4-Tetramethylbenzene	1.67	2.63	1.97	4.37	92.96	3.661
1,2,3,5-Tetramethylbenzene	1.77	2.64	1.94	4.37	92.96	3.655
1,2,4,5-Tetramethylbenzene	1.75	2.77	1.82	4.37	92.96	3.655
sec. Amylbenzene	2.52	3.87	2.34	4.71	100.42	4.392
tert. Amylbenzene	2.21	3.45	2.25	4.64	100.41	4.221
Mobile phase concentration: acetonitrile/water	70/30	45/55	75/25			

* log P = hydrophobic parameter; V_W = van der Waals volume; χ = molecular connectivity index

Table II. Relationship between the logarithm of the capacity factor and the various physical parameters for alkylbenzenes on reversed-phase columns

physical parameter	correlation coefficients					
	our measurements*			literature values**		
	C_2	C_8	C_{18}	C_2	C_{18}	C_{22}
hydrophobic parameter (log P)	0.88697	0.91902	0.91604	0.85548	0.93177	***0.94964***
van der Waals volume (V_W)	0.90621	0.93197	***0.92454***	0.89559	***0.93611***	0.94193
molecular connectivity index (χ)	***0.93121***	***0.93903***	0.84321	***0.91672***	0.85665	0.80798
number of compounds	19	19	19	11	11	11

* See Table I

** Data of Smith [30] for the same solutes as investigated by us.

Table III. Relationship between the capacity factors obtained on two columns

Columns	Correlation coefficient
Our measurements	
C_2 vs. C_8	0.98925
C_2 vs. C_{18}	0.89569
C_8 vs. C_{18}	0.93004
Data of Smith [30]	
C_2 vs. C_{18}	0.93415
C_2 vs. C_{22}	0.91832
C_{18} vs. C_{22}	0.99185

Table IV. Relationship between the logarithm of the capacity factor of alkylbenzenes measured on reversed-phase columns and physical parameters of the solutes, with multi-regression analysis.

Columns	Correlation coefficient**		
	1**	2**	3***
Our measurements			
C_2	χ : 0.93121	V_W : 0.94430	logP: 0.95162
C_8	χ : 0.93903	V_W : 0.96001	logP: 0.96218
C_{18}	V_W: 0.92454	logP: 0.92523	χ : 0.92578
Data of Smith [30]			
C_2	χ : 0.91672	V_W : 0.94433	logP: 0.94523
C_{18}	V_W : 0.93511	χ : 0.94195	logP: 0.94881
C_{22}	logP: 0.94964	χ : 0.95403	V_W : 0.95509

* χ = molecular connectivity index; V_W = van der Waals volume; log P = hydrophobic parameter.

** The number means the order of the dominant for each correlation.

It is apparent from the results that the interaction between the solutes and the C_2 stationary phase is partially dissimilar to the interaction between the solutes and the C_{18} stationary phase.

Simultaneous correlation with physical parameters

From this discussion, it is clear that the size, shape and hydrophobicity of the molecules of alkylbenzenes contribute to the control of the retention in RPLC. Therefore, multi-regression analyses were carried out for each data set to improve the correlation coefficients between $\log k'$ and the three physical parameters.

The results are given in Table IV. It is apparent that multi-regression analysis for stationary phases having shorter alkyl-chains are effective to indicate more accurately the correlation between $\log k'$ and the physical parameters. For stationary phases having longer alkyl-chains the opposite is observed. This conclusion can be drawn from the facts that the improvements in the correlation coefficients with multi-regression analyses for C_2 and C_8 columns are superior to those for C_{18} and C_{22} columns.

Table V gives the equations we could derive to describe the simultaneous relationship between $\log k'$ and the physical parameters of alkylbenzenes, for two columns, together with the results of Smith [30] for a C_2 column. The number of data used and the correlation coefficients obtained for these equations by regression analysis are also given. It is obvious that these equations describing the simultaneous relationships have the highest correlation coefficient among all the relationships studied.

Table V. Relationship between the logarithm of the capacity factor of alkyl'benzenes measured on reversed-phase columns and physical parameters of the solutes, for columns containing stationary phases with shorter alkyl chains.

Column	Equation	n*	correlation coefficient
C_2	$\log k' = 0.13498\,\chi + 0.01260\,V_W - 0.19078\,\log P - 0.58125$	19	0.95162
C_8	$\log k' = 0.12610\,\chi + 0.01072\,V_W - 0.11548\,\log P - 0.51156$	19	0.96218
C_2**	$\log k' = 0.26407\,\chi + 0.01778\,V_W - 0.14219\,\log P - 1.15525$	11	0.94523

* number of componds used in setting up the equations.

** data of Smith [30].

Conclusions

Good correlation was found on stationary phases with shorter alkyl chains between the log k′ values of alkylbenzenes and the three physical parameters indicating size, topological shape and hydrophobicity of the solute molecules. In the case of stationary phases with longer alkyl chains, good correlation was found between the log k′ values of alkylbenzenes and either their van der Waals volume (V_w) or the hydrophobic parameter (log P). These relationships may be useful in the prediction of the retention of alkylbenzenes in RPLC.

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