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ENERGY EFFECTS IN THE RETENTION OF AROMATIC ACIDS IN LIQUID CHROMATOGRAPHY

T. HANAI

Gasukuro Kogyo Inc., 237-2 Sayamagahara, Iruma 358 (Japan)

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SUMMARY

The reversed-phase liquid chromatographic behaviour of phenols and aromatic acids was studied using an octadecyl-bonded silica gel as the stationary phase and acidic acetonitrile–water mixtures as the mobile phase. The retentions of these compounds can be predicted from their Van der Waals volumes and energy effects. The energy effects are classified according to π and hydrogen-bonding energies. The inclusion of dissociation constants, derived from Hammett's equation, in the above approach makes it possible to predict the retention times of ionized aromatic acids.

INTRODUCTION

Several approaches have been used to develop systems for the prediction of retention times in reversed-phase liquid chromatography. These have included alkyl chain length, connectivity index, number of double bonds, localization and delocalization energies, Hansch's π constants and Rekker's hydrophobic fragmental constants. Of these parameters, Rekker's hydrophobic fragmental constants ($\log P$ values)¹ were useful for predicting the retention times of several groups of compounds in systems with octadecyl-bonded silica gels as the stationary phase and pH-controlled acetonitrile–water mixtures as the mobile phase^{2–5}.

However, each group of compounds required individual standard compounds in order to obtain the constants of the equations that were used for the calculation of the retention time, the resolution and the concentration of acetonitrile as an organic modifier. This meant that if a compound had two or more different types of substituents, the retention time predicted from one set of equations was often far from the observed value. The system based on the partition coefficient between octanol and water was, therefore, not adequate for producing an optimized system for mixtures of different types of compounds. Further investigations have used basic physico-chemical parameters such as Van der Waals volumes, π -energies, hydrogen-bonding energies and dissociation constants. In systems with octadecyl-bonded silica gels and pH-controlled acetonitrile–water mixtures, the retention times of non-ionizable compounds, such as *n*-alkanes, polyaromatic hydrocarbons, alkylbenzenes and halogenated benzenes, have been well predicted from the Van der Waals volume and

π -energy effects^{6,7}. The retention of phenols has been calculated from the following equation:

$$\log k'(\text{Ar-OH}) = \log k'(\text{VWV}) - \log k'(\pi) - \log k'(\text{HB})$$

where $\log k'(\text{Ar-OH})$ is the capacity ratio of a substituted phenol, $\log k'(\text{VWV})$ is obtained from the Van der Waals volume, $\log k'(\pi)$ is the π -energy effect of a phenyl group and $\log k'(\text{HB})$ is the hydrogen bonding effect of the hydroxy group of phenol⁸. This approach has now been applied to produce an optimization system for aromatic acids in liquid chromatography.

EXPERIMENTAL

The details of the instrument used were described previously⁵ and the chemicals used are listed in Table I. The octadecyl-bonded silica gel column was an ERC-1000, kindly donated by ERMA Optical Works (Tokyo, Japan). The column temperature was 40°C. The Van der Waals volumes were calculated by Bondi's method⁹ and the values of the energy effect of phenols were obtained from a previous paper⁸.

RESULTS AND DISCUSSION

The $\log k'$ values measured are listed in Table I together with the values of the energy effect. The relationship between $\log k'$ and Van der Waals volumes was linear. However, the slopes for the different groups of compounds were not exactly the same, *e.g.*, hydrophobic groups had higher slopes. The difference in slopes could be explained by enthalpy effects¹⁰. The energy effect of each compound could easily be calculated if a linear relationship between Van der Waals volumes and $\log k'$ values for *n*-alkanes could be obtained. However, the retention times of *n*-alkanes were very long with eluents containing low concentrations of an organic modifier. Thus, such plots could not be obtained easily for aromatic acids, the retention times of which were relatively short. Therefore, a linear relationship for alkylphenols between their $\log k'$ values and their Van der Waals volumes was first obtained. The capacity ratios of *n*-alkanes were then calculated from their Van der Waals volumes and from the values of the energy effect of phenols.

The slope of the linear relationship for *n*-alkanes was further adjusted by the addition of the enthalpy effect¹⁰. The difference between the observed energy effect of phenols and the reference values was less than 5%. The energy effect of aromatic acids was obtained from the last equation and their $\log k'$ values. The values are given in Table I.

The hydrogen-bonding energy effect of a carboxyl group of benzoic acid is about 2 kcal/mole higher than that of a phenolic hydroxy group. The difference between the energy effects of benzoic acid and phenylacetic acid is about 2 kcal/mole, and that between phenylacetic acid and 3-phenylpropionic acid or 4-phenylbutyric acid is small. A similar result was obtained for indole acids. In addition, the retention times of ionized aromatic acids were predicted from their $\log P$ values and their dissociation constants^{11,12}. This equation was modified to

$$k' = (k'_{\max} + k'_{\min} K_a / [\text{H}^+]) / (1 + K_a / [\text{H}^+])$$

where the maximum capacity ratios (k'_{\max}) were calculated from their Van der Waals volumes and their energy effects instead of their $\log P$ values. The $\text{p}K_a$ values were calculated from a modified Hammett equation¹². The minimum capacity ratios (k'_{\min}), *i.e.*, the capacity ratios of totally ionized acids, were close to zero and could not be predicted, so the values were obtained experimentally in the eluent of pH 7.000. The result is shown in Fig. 1.

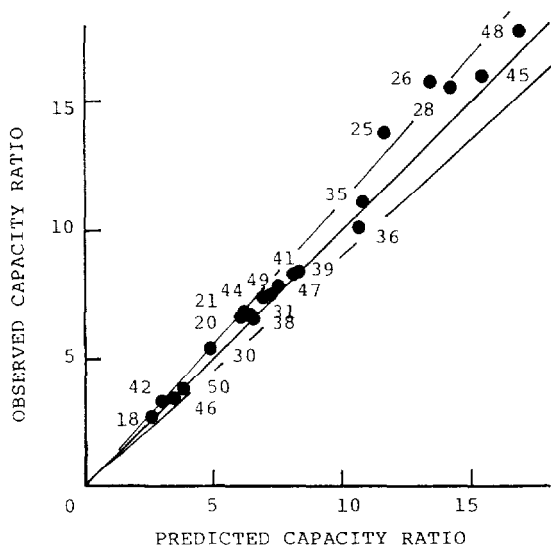


Fig. 1. Predicted and observed capacity ratios of aromatic acids at pH 4.500. Experimental conditions: column, ERC-1000 (ODS), 15 cm \times 6.0 mm I.D.; eluent, 0.05 M sodium phosphate (pH 4.500) containing 25% of acetonitrile; column temperature, 40°C. The numbers adjacent to the symbols are the same as in Table I. The two lines on either side of the central line indicate a 10% error ($n = 21$).

The difference between the predicted and observed capacity ratios was 10%. The error for 3,4- and 3,5-dimethylbenzoic acids (Nos. 25 and 26 in Fig. 1) was more than 10%, but the error for 3,4-dimethylbenzoic acid should depend on the value of the π -energy effect but not the dissociation constant.

The following equation was obtained between the predicted and observed capacity ratios:

$$k'_{\text{obs}} = a/k'_{\text{pre}} + b$$

where k'_{obs} and k'_{pre} are the observed and predicted capacity ratios, respectively, and constants a and b are 1.132 and -0.2038 , respectively. The correlation coefficient ($n = 21$) was 0.990 and the average error was 4.78%. Only 3,5-dichlorobenzoic acid (No. 36 in Fig. 1) had an error of more than 10%. However, the error for this acid at pH 1.900 was very small, and therefore should be due to the dissociation constant.

TABLE I

COMPOUNDS STUDIED AND THEIR PHYSICAL PARAMETERS

Experimental conditions: column, ERC-1000 (ODS), 15 cm \times 6.0 mm I.D.; eluent, 0.05 M phosphoric acid in acetonitrile-water mixtures; column temperature, 40°C.

Compound	VW/V^*	E^{**}	pK_a^{***}	$\log k'$ % acetonitrile					E^{\S}
				20	25	30	35	40	
1 Phenol	53.88	7.883	—	—	0.5186	—	0.2738	—	7.963
2 2-Methylphenol	65.03	8.259	—	1.086	0.8723	0.6924	0.5691	0.4596	8.410
3 3-Methylphenol	65.03	—	—	1.040	0.8242	0.6932	0.5137	0.4047	8.643
4 4-Methylphenol	65.03	8.704	—	1.038	0.8248	0.6922	0.5094	0.4027	8.653
5 2,3-Dimethylphenol	76.18	9.116	—	1.393	1.143	0.9872	0.7851	0.6526	9.197
6 2,4-Dimethylphenol	76.18	—	—	1.427	1.172	1.013	0.8058	0.6713	9.057
7 2,5-Dimethylphenol	76.18	9.013	—	1.427	1.172	1.015	0.8127	0.6734	9.045
8 2,6-Dimethylphenol	76.18	8.524	—	1.409	1.174	1.026	0.8320	0.7047	8.984
9 3,4-Dimethylphenol	76.18	9.762	—	1.320	1.067	0.9725	0.7007	0.5714	9.570
10 3,5-Dimethylphenol	76.18	9.489	—	1.385	1.126	0.9615	0.7487	0.6179	9.337
11 2,3,5-Trimethylphenol	87.33	9.851	—	1.735	1.449	1.259	1.020	0.8979	9.844
12 2,3,6-Trimethylphenol	87.33	9.360	—	1.717	1.454	1.270	1.047	0.8986	9.844
13 2,4,6-Trimethylphenol	87.33	9.175	—	1.758	1.487	1.303	1.072	0.9208	9.644
14 2,3,5,6-Tetramethylphenol	98.48	10.13	—	—	—	—	1.263	1.092	10.46
15 2-Ethylphenol	75.26	9.192	—	1.487	1.232	0.9985	0.8647	0.7259	8.627
16 3-Ethylphenol	75.26	—	—	1.490	1.155	0.9902	0.7800	0.6426	8.946
17 4-Ethylphenol	75.26	9.122	—	1.432	1.168	0.9994	0.7858	0.6466	8.946
18 Benzoic acid	65.36	—	4.200	0.8271	0.6065	0.4489	0.2794	0.1557	10.00
19 2-Methylbenzoic acid	76.51	—	—	1.140	0.8853	0.7058	0.5117	0.3712	10.76
20 3-Methylbenzoic acid	76.51	—	4.270	1.216	0.9444	0.7507	0.5399	0.3957	10.51
21 4-Methylbenzoic acid	76.51	—	4.340	1.177	0.9263	0.7516	0.5248	0.3803	10.61
22 2,4-Dimethylbenzoic acid	87.66	—	—	1.535	1.228	1.007	0.7733	0.6084	11.27
23 2,5-Dimethylbenzoic acid	87.66	—	—	1.356	1.231	1.007	0.7715	0.6092	11.43
24 2,6-Dimethylbenzoic acid	87.66	—	—	1.137	0.8348	0.7064	0.5068	0.3740	13.00
25 3,4-Dimethylbenzoic acid	87.66	—	4.440	1.225	1.209	0.9072	0.7315	0.5674	11.62
26 3,5-Dimethylbenzoic acid	87.66	—	4.340	1.456	1.288	1.050	0.8032	0.6334	11.16
27 2,4,6-Trimethylbenzoic acid	98.81	—	—	1.527	1.228	1.011	0.7782	0.6125	13.44
28 4-Ethylbenzoic acid	86.74	—	4.350	1.598	1.284	1.049	0.8090	0.6310	10.85

29	2-Chlorobenzoic acid	74.84	—	—	0.9933	0.7629	0.5878	0.4075	0.2691	11.09
30	3-Chlorobenzoic acid	74.84	—	3.380	1.011	1.082	0.7626	0.6548	0.5016	9.715
31	4-Chlorobenzoic acid	74.84	—	3.960	1.370	1.097	0.8897	0.6700	0.5135	9.419
32	2,4-Dichlorobenzoic acid	84.32	—	—	1.587	1.289	1.067	0.8306	0.6585	10.30
33	2,5-Dichlorobenzoic acid	84.32	—	—	1.489	1.206	0.9944	0.7687	0.6032	10.70
34	2,6-Dichlorobenzoic acid	84.32	—	—	1.049	0.8365	0.6796	0.4977	0.3669	12.48
35	3,4-Dichlorobenzoic acid	84.32	—	3.600	1.847	1.517	1.258	0.9914	0.8063	9.223
36	3,5-Dichlorobenzoic acid	84.32	—	3.460	1.929	1.588	1.338	1.077	0.8818	8.791
37	2-Bromobenzoic acid	77.96	—	—	1.077	0.8317	0.6490	0.4595	0.3215	11.35
38	3-Bromobenzoic acid	77.96	—	3.810	1.470	1.175	0.9595	0.7300	0.5647	9.641
39	4-Bromobenzoic acid	77.96	—	3.980	1.505	1.195	0.9792	0.7475	0.5821	9.522
41	2-Chlorophenylacetic acid	85.07	—	—	1.188	0.9203	0.7209	0.5165	0.3676	12.34
42	4-Chlorophenylacetic acid	85.07	—	4.216	1.364	1.081	0.8601	0.6359	0.4739	11.57
42	Phenylacetic acid	79.55	—	4.300	—	0.6309	—	—	—	12.88
43	2-Tolylacetic acid	86.74	—	—	1.144	0.8849	0.6904	0.4916	0.3478	12.83
44	3-Tolylacetic acid	86.74	—	4.325	1.233	0.9625	0.8761	0.5503	0.4017	12.51
45	4-Phenyl- <i>n</i> -butylic acid	96.05	—	4.719	1.517	1.212	0.9807	0.7441	0.5714	13.05
46	L(+)-Mandelic acid	80.18	—	3.380	0.2384	0.0492	-0.0804	-0.2516	-0.3393	16.03
47	<i>trans</i> -Cinnamic acid	82.32	—	4.380	1.263	0.9754	0.7629	0.5476	0.3906	11.56
48	4-Methylcinnamic acid	93.47	—	4.492	1.650	1.314	1.060	0.8171	0.6241	12.08
49	3-Phenyl- <i>n</i> -propionic acid	86.04	—	4.579	1.190	0.9200	0.7323	0.5281	0.3782	12.49
50	Indole-3-acetic acid	91.65	—	4.750	0.8907	0.6270	0.2058	0.2384	0.1023	15.42
51	Indole-3-propionic acid	114.11	—	—	1.214	0.9130	0.6889	0.4650	0.3003	18.19
52	Indole-3-butylic acid	121.34	—	—	1.492	1.153	0.8953	0.6511	0.4584	18.44
53	Hippuric acid	96.15	—	—	0.2225	0.0116	-0.1430	-0.3132	-0.4259	19.31
	Void volume (elution volume of Na ₂ NO ₃ , ml)				1.950	1.992	1.860	1.926	1.800	
	Dead volume of instrument (ml)				0.067	0.116	0.067	0.116	0.067	

* Van der Waals volume calculated by Bondi's method⁹.

** Energy effect (kcal/mol) from ref. 8.

*** Dissociation constant from ref. 12.

§ Energy effect obtained from the observed log *k'* values.

CONCLUSION

The retention times of aromatic acids with an eluent of given pH can be obtained from their Van der Waals volumes, energy effects and dissociation constants. The remaining difficulty is the substituent effect in estimating the energy effect. Hammett's σ and Taft's σ^* constants did not bear a linear relationship to their $\log k'$ values. Hammett's σ constant was useful only for calculating the dissociation constant, even though the estimated dissociation constants for multi-substituted compounds were uncertain because not many data have been reported.

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