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Journal of Chromatography A, 797 (1998) 23–31

JOURNAL OF CHROMATOGRAPHY A

Comparison of retention models for the dependence of retention factors on mobile phase composition in reversed-phase high-performance liquid chromatography

Kyung Ho Row

Department of Chemical Engineering, Inha University, 253 Yonghyun-Dong, Nam-ku, Inchon 402-751, South Korea

Abstract

Chromatographic retention behavior of five deoxyribonucleosides (dCyd, dUrd, dGuo, dThd and dAdo) with respect to the mobile phase composition was studied under isocratic conditions of reversed-phase high-performance liquid chromatography (RP-HPLC). The volume fraction (F) of organic modifier was changed from 0.05 to 0.30, and to 0.12 for methanol and acetonitrile, respectively. The experimental data of nitro and steroid compounds were also considered for comparison of five retention models with various classes of samples. The Langmuir-type retention model ($k'=A+B/F$) with the two parameters, A and B, shows excellent agreement between the experimental retention factors and calculated values although the values by the log-scale quadratic model with the three parameters ($\log k'=LF^2+MF+N$) are closer. Unlike other retention models, the slope, B, of the Langmuir-type retention model can characterize the properties of solute and organic modifier simultaneously. For each solute, the intercept, A, calculated for acetonitrile and methanol as organic modifiers are coincident close. © 1998 Elsevier Science B.V.

Keywords: Retention models; Retention factor; Mobile phase composition; Deoxyribonucleosides; Steroids; Nitro compounds

1. Introduction

As high-performance liquid chromatography (HPLC) is widely used as a standard analytical technique, a number of stationary phases are commercially available. HPLC columns are improved to increase the selectivity and the efficiency for the mixtures separated. The most commonly used technique is reversed-phase (RP) HPLC, which is usually carried out with *n*-octadecyl modified packings [1]. As C_{18} is chemically bonded to the surface of the particle, these packings provide stability and reproducibility as well as selectivity [2].

Five nitrogenous bases are found in DNA and RNA nucleotide components. Three of the bases, adenine, guanine and cytosine, are common to DNA and RNA. Thymine is found only in DNA, while uracil is unique to RNA. Adding the bases to deoxyribose five-carbon sugar results in the formation of deoxyribonucleoside. In recent years, use of HPLC techniques to analyze the DNA fragments have significantly increased.

The important parameter for quantitation in HPLC is the retention factor (k'). Retention volume of a sample compound (V_R) can be expressed in terms of the elution volume of a nonretained material (V_0). k' is defined by the ratio of (V_R-V_0) to V_0. The retention factor is proportional to the free energy change associated with the chromatographic distribution process. It is also related to the partition coefficient. Thus, solute retention is affected by the thermodynamics of distribution between the stationary and

0021-9673/98/$19.00
PII S0021-9673(97)00969-2

mobile phases. The compositions of mobile phase determine the retention volume of solutes. For RP-HPLC column, the major constituent is a highly polar solvent (e.g., water), and the less polar solvents of organic modifier (e.g., methanol, acetonitrile, etc.) are added to control the hydrophobic nature between solute and C_{18}-coated stationary phase. Snyder's equation has been typically used to describe the logarithmic relationship between k' and the fraction of mobile phase [3]. But recently, the more elaborate equation based on the adsorption of Langmuir adsorption shows better prediction of k' with different composition of mobile phase [4]. For the nitro and steroid samples from the literature [3] as well as the solutes of deoxyribonucleosides experimentally obtained, five retention models including the Langmuir-type retention model and the Snyder's equation were compared with the experimental data. Therefore, the purpose of the work is to compare the five retention models to predict the retention factors for nitro compounds and steroid compounds as well as deoxyribonucleoside, and the differences in the retention mechanisms will be discussed for the solutes.

2. Retention mechanisms

Normally, the prediction of retention time is based on some expected dependence of retention factor, k', on mobile phase composition. Retention volume may be expressed as retention time at the constant flow-rate of mobile phase. More often, the problem of extrapolation of experimental data to estimate the value of retention factor for water as mobile phase (k'_w) is discussed in the literature [3]. The value of k'_w serves as a good descriptor and predictor of the solute hydrophobicity in biological systems [5].

Snyder described the following linear relationship in RP-HPLC [3]:

$$\log k' = \log k'_w - SF \tag{1}$$

where k' refers to the solute retention factor, k'_w is the value of k' for water as mobile phase, F is the volume fraction of organic modifier in the mobile phase and S is a constant for a given solute. The slope and intercept values of Eq. (1) are regarded as a measure of the hydrophobic character of the solutes [6]. The considerable amount of literature reported the use of Eq. (1) for the estimation of the retention of solutes in RP-HPLC, and some are discussed and reviewed in detail [2,3,5–10].

Due to the dependence of log k' on the mobile phase composition, attempts have been made to find an alternative chromatographic parameter that is less dependent on the conditions and can be used as a continuous and universal scale. Hsieh and Dorsey [11] suggested the following form:

$$\log k' = K \log (1/F) + H \tag{2}$$

where K and H are empirical coefficients.

The simple polynomial of quadratic form is adopted and the two types of k', normal and log scale is as follows,

$$k' = CF^2 + DF + E \tag{3}$$

$$\log k' = LF^2MF + N \tag{4}$$

where C, D, E, L, M and N are empirical coefficients.

Finally, the Langmuir-type relationship between retention factor and organic modifier content in a mobile phase was first proposed by Row and co-workers [4,10]. This equation assumed that the adsorption of organic modifier is described by Langmuir isotherm. The final equation can be expressed as follows:

$$k' = A + B(1/F) \tag{5}$$

where A and B are experimental coefficients. The intercept, A, characterizes the adsorption interaction between the organic modifier molecules and adsorbent surface while the slope, B, relates to the solute molecules and adsorbent surface interaction. Unlike the other four equations, Eq. (5) was theoretically developed with a few assumptions [4,10].

All equations were linearized by Lotus 123 (Ver. 2.0). The resulting correlation coefficients, r^2, have the following form,

$$r^2 = \frac{\left[\sum (x_i - \bar{x})(y_i - \bar{y})\right]^2}{\left[\sum (x_i - \bar{x})^2\right]\left[\sum (y_i - \bar{y})^2\right]} \tag{6}$$

3. Experimental

All deoxyribonucleosides were chromatographically pure and were purchased from Sigma (St. Louis, MO, USA). The solutes were dissolved in HPLC-grade water and each concentration was 50 μg/ml.

HPLC-grade water, methanol and acetonitrile were obtained from Baker (Phillipsburg, NJ, USA). Waters Model 600 liquid chromatograph [Waters Associates, Milford, MA, USA equipped with a Waters 600E Multisolvent Delivery System, a UV–visible tunable wavelength absorbance detector (Waters 486) and a U6K injector (2 ml sample loop] was used. The data acquisition system was Chromate (Ver. 2.l, Interface Eng.) installed in a personal computer. A Waters column (30×0.39 cm) packed by μBondapak C_{18} reversed-phase material of 10 μm particle size was used.

The modifier concentrations of methanol and acetonitrile ranged from 0 to 30% and from 0 to 12% (v/v), respectively. The injection volume of 5 μl was injected directly for HPLC analysis. The elutions were performed by using an isocratic mode at a flow-rate of 1 ml/min. Absorbance was monitored at 254 nm with a sensitivity of 2 and 0.001 a.u.f.c. All separations were done at the ambient temperature. The dead volume was measured by introducing 20 μl of methanol, and it was 2.95 ml.

4. Results and discussion

The experimentally measured retention factors of the five deoxynucleosides in water–methanol and water–acetonitrile mobile phases with respect to the content of organic modifier are presented in Figs. 1 and 2, respectively. The experimental data of nitro and steroid compound were also added in the Figures to compare the retention models with various classes of sample [3]. The experimental data of deoxyribonucleosides were characterized by the lower content of organic modifier, while Snyder's data of the nitro and steroid samples by the higher content of organic modifier. In the both cases, the retention times of deoxyribonucleosides, nitro and steroid compounds decrease with an increase in the con-

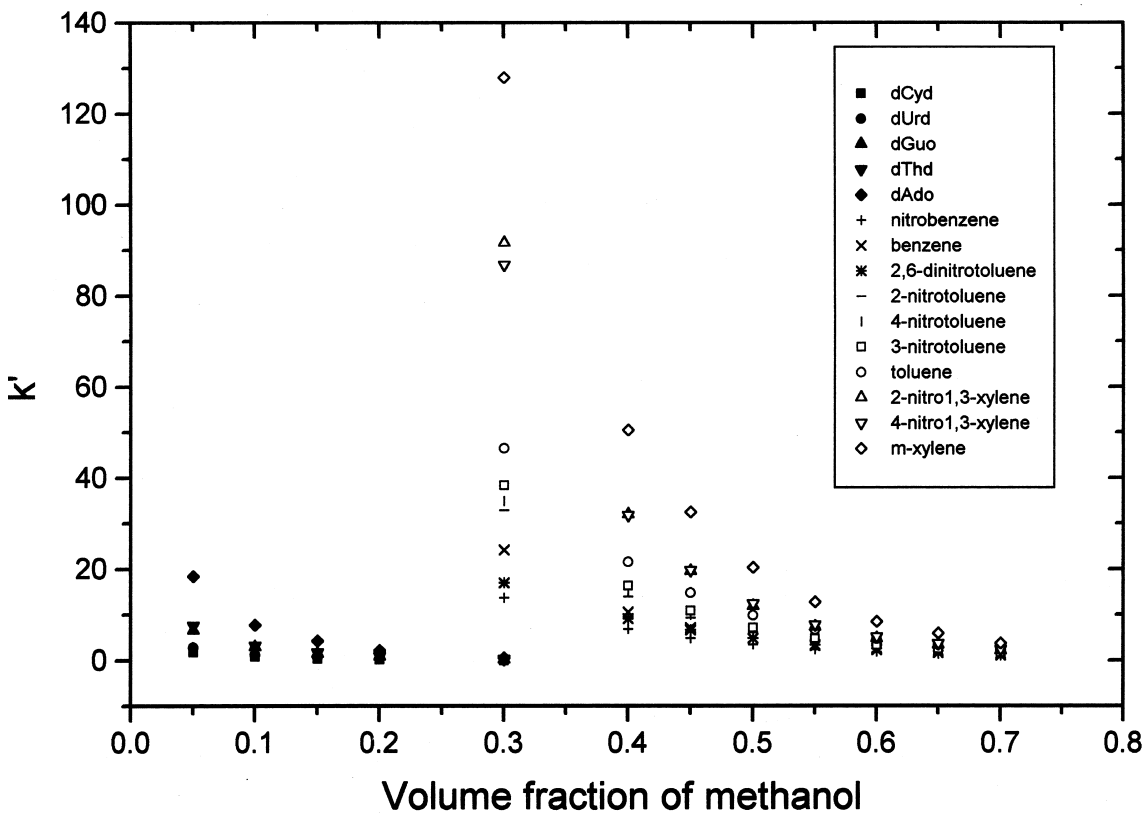

Fig. 1. Effect of volume fraction of methanol on k'.

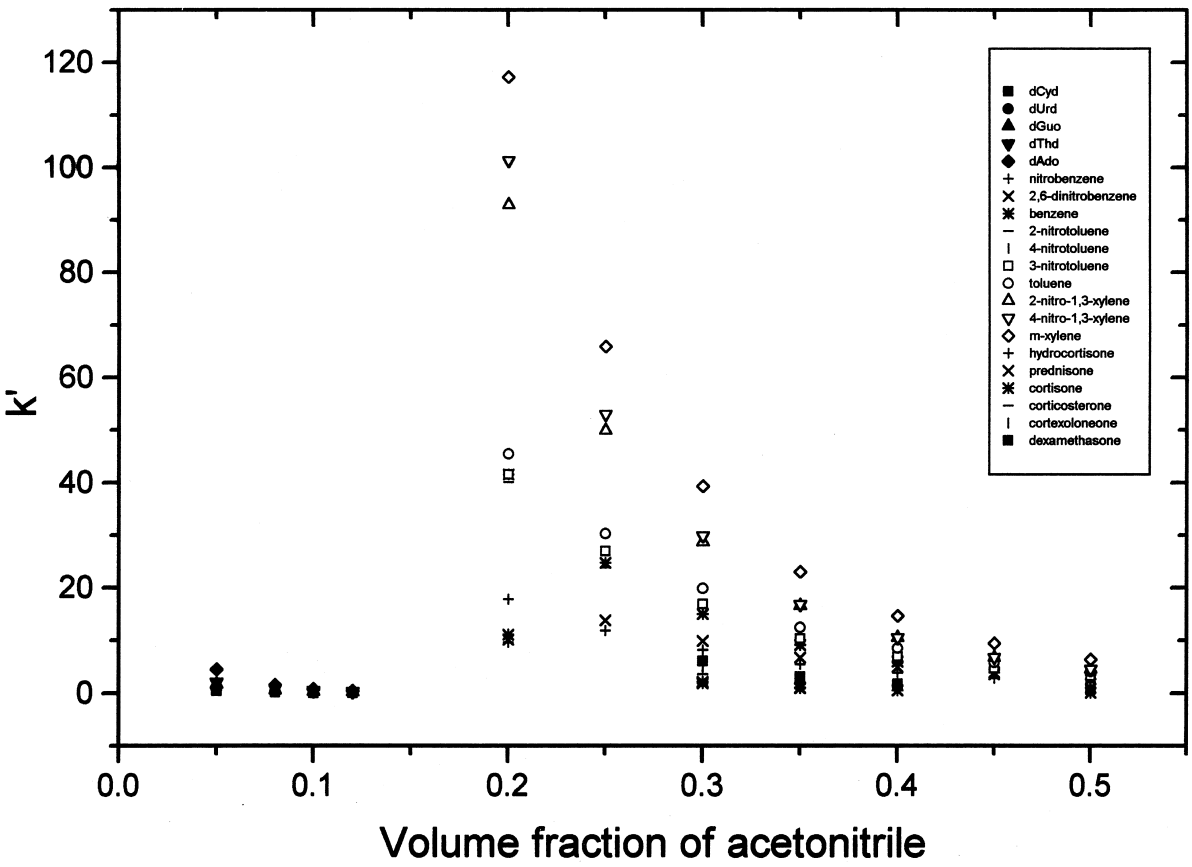

Fig. 2. Effect of volume fraction of acetonitrile on k'.

centration of modifier in a semi-logarithmic relationship. Compared to methanol, acetonitrile offers approximately twice the elution power for nucleosides, but there is no significant difference in the separation selectivity. The elution order of deoxynucleosides is the same in the different mobile phases (Figs. 1 and 2). The retention values increase as the following: dCyd–dUrd–dThd–dGuo–dAdo. Here, dCyd, dUrd and dThd contain the pyrimidine bases, and dGuo and dAdo contain the purine bases. This result can be related with the increase of molecule size and, therefore, the increase of the surface area of a solute molecule [7]. The retention order on the Bondapak column in pure water probably corresponds to the hydrophobicity of the investigated deoxynucleosides, i.e., their affinity for this surface.

k'_w is the value of retention factor for water only as a mobile phase. In RP-HPLC without the organic modifier in mobile phase, the retention time of sample is very long because water is passed through but the sample is retained on the hydrophobic C_{18} surface. The k'_w value can be obtained by extrapolation from the experimental dependence of log k' vs. organic modifier content. As shown in Fig. 3, the dependence of the log k'_w intercepts calculated for acetonitrile vs. the log k'_w intercepts calculated for methanol as organic modifiers is fitted by straight line arising from origin. That is, the intercepts of Eq. (1) calculated by using the experimental values of log k'_w for different organic modifiers are slightly dependent. The intercepts calculated are independent on the nature of organic modifier. The extrapolated values of log k'_w measured in the regions of different

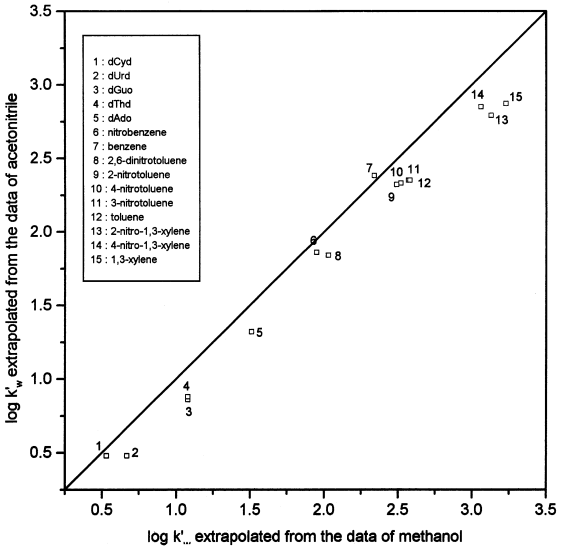

Fig. 3. Comparison of the experimental values of k' extrapolated from the data of methanol and acetonitrile.

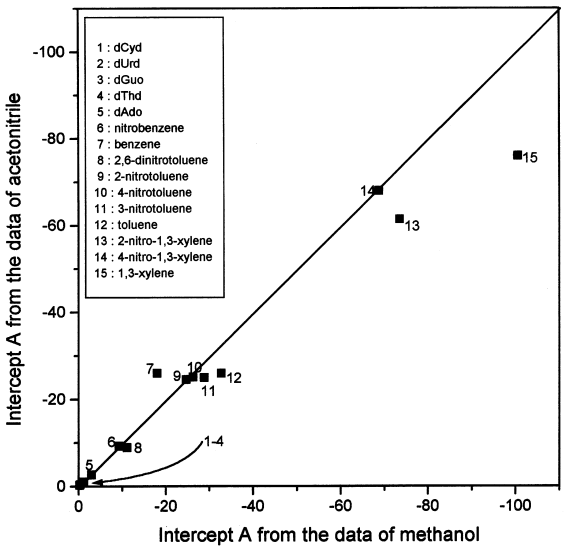

Fig. 4. Comparison of the intercept, A, of Eq. (5) from the data of methanol and acetonitrile.

content of organic modifier appear to be in the functional dependence on hydrophobic parameters of solutes.

The dependence of k' vs. $1/F$ plots are characterized by the different magnitudes of slopes for each deoxyribonucleoside. In the following Langmuir-type relationship, i.e., Eq. (5), the intercept, A, and the slope, B, were obtained by the regression analysis for the five deoxyribonucleosides and 10 nitro-compounds. These results are illustrated in Fig. 4 by straight line arising from origin. As shown in Fig. 4, the intercepts calculated for acetonitrile and for methanol as organic modifiers are coincident. Specially, for the deoxyribonucleosides with the lower content of the organic modifiers, the values very similar. At the higher content of the organic modifiers, the data points are slightly deviated from the diagonal line. Table 1 indicates that the ratios of the slopes are greatly divided by the type of sample. The table also shows that their ratios $B_{\text{methanol}}/B_{\text{acetonitrile}}$ of deoxyribonucleosides are changed in a comparatively narrow range from 2.750 to 3.000, but the ratios of nitro compounds are changed in a wider range from 0.928 to 1.753. Therefore, in the small content of organic modifier, the ratios of slopes can characterize the properties of organic modifier only. As the content of organic modifier in mobile phase increases, the slopes are affected by both the sample and the organic modifier simultaneously.

Linear regression was carried out according to Eqs. (1)–(5) for each solute (deoxyribonucleosides, nitro and steroid compounds) and organic modifier (methanol and acetonitrile). The slopes, intercepts, and correlation coefficients calculated are listed in Tables 2 and 3 for the organic modifier of methanol and acetonitrile, respectively. The retention factors of six steroid samples are listed with acetonitrile only in Table 3. For deoxyribonucleosides, Langmuir-type

Table 1
Ratio of slopes calculated for methanol and acetonitrile by Eq. (5)

Materials	$B_{\text{MeOH}}/B_{\text{ACN}}$
dCyd	2.750
dUrd	2.833
dGuo	3.000
dThd	2.750
dAdo	2.972
Nitrobenzene	1.253
Benzene	0.928
2,6-Dinitrotoluene	1.468
2-Nitrotoluene	1.322
4-Nitrotoluene	1.359
3-Nitrotoluene	1.472
2-Nitro-1,3-xylene	1.580
4-Nitro-1,3-xylene	1.368
m-Xylene	1.753

Table 2
Calculated results of the parameters used in Eqs. (1)–(5) in organic modifier of methanol

Materials	Parameters																	
	Eq. (1)			Eq. (2)			Eq. (3)				Eq. (4)				Eq. (5)			No. of data points
	Log k'_w	S	r^2	H	K	r^2	C	D	E	r^2	L	M	N	r^2	A	B	r^2	
dCyd	0.53	−5.64	0.9977	−1.90	1.17	0.937	42.88	−21.83	2.79	0.9788	1.39	−6.13	0.57	0.9981	−0.27	0.11	0.9965	5
dUrd	0.67	−5.27	0.9933	−1.63	1.67	0.9595	67.67	−33.91	4.27	0.9662	4.39	−6.83	0.77	0.9976	−0.42	0.17	0.9990	5
dGuo	1.08	−5.84	0.9977	−1.45	1.84	0.9453	158.39	−79.37	9.91	0.9732	2.54	−6.73	1.14	0.9989	−1.06	0.39	0.9992	5
dThd	1.08	−5.29	0.9902	−1.24	1.69	0.9683	183.49	−91.03	11.30	0.9658	6.13	−7.46	1.22	0.9984	−1.17	0.44	0.9999	5
dAdo	1.51	−5.74	0.9963	−0.98	1.81	0.9497	440.42	−219.94	27.36	0.9669	3.25	−6.89	1.59	0.9983	−2.96	1.07	0.9997	5
Nitrobenzene	1.95	−2.79	0.9973	−0.41	3.06	0.9913	99.41	−129.18	43.19	0.9925	1.00	−3.79	2.19	0.9993	−9.30	6.68	0.9793	8
Benzene	2.34	−3.28	0.9978	−0.44	3.60	0.9914	201.76	−254.90	81.71	0.9865	1.11	−4.40	2.61	0.9996	−17.98	12.06	0.9607	8
2,6-Dinitrobenzene	2.03	−2.66	0.9987	−0.23	2.91	0.9867	112.27	−149.50	51.53	0.9960	0.46	−3.13	2.14	0.9992	−11.15	8.28	0.9889	8
2-Nitrotoluene	2.49	−3.35	0.9970	−0.35	3.68	0.9926	281.55	−353.58	112.42	0.9852	1.36	−4.72	2.81	0.9995	−24.69	16.40	0.9555	8
4-Nitrotoluene	2.52	−3.36	0.9972	−0.32	3.68	0.9923	298.90	−375.51	119.43	0.9852	1.31	−4.67	2.83	0.9995	−26.25	17.44	0.9558	8
3-Nitrotoluene	2.57	−3.38	0.9974	−0.29	3.71	0.9921	328.67	−412.86	131.26	0.9853	1.27	−4.66	2.87	0.9996	−28.89	19.17	0.9557	8
Toluene	2.63	−3.24	0.9991	−0.03	3.37	0.9509	357.93	−458.66	150.21	0.9770	0.65	−3.90	2.78	0.9998	−34.21	23.25	0.9703	8
2-Nitro-1,3-xylene	3.13	−4.04	0.9961	−0.30	4.44	0.9938	903.40	−1102.68	336.45	0.9734	1.89	−5.95	3.58	0.9994	−73.53	46.11	0.9203	8
4-Nitro-1,3-xylene	3.06	−3.87	0.9967	−0.22	4.25	0.9936	830.78	−1019.93	313.82	0.9760	1.71	−5.59	3.46	0.9997	−68.75	43.61	0.9288	8
m-Xylene	3.23	−3.81	0.9987	−0.01	4.17	0.9893	1167.49	−1449.86	452.57	0.9821	0.94	−4.75	3.45	0.9997	−100.58	64.65	0.9442	8

Table 3
Calculated results of the parameters used in Eqs. (1)–(5) in organic modifier of acetonitrile

Materials	Parameters																	
	Eq. (1)			Eq. (2)			Eq. (3)				Eq. (4)				Eq. (5)			No. of data points
	$\log k_w$	S	r^2	H	K	r^2	C	D	E	r^2	L	M	N	r^2	A	B	r^2	
dCyd	0.48	−14.64	0.9870	−3.62	2.61	0.9453	66.69	−18.00	1.25	0.9968	−61.03	−4.35	0.09	0.9949	−0.28	0.04	0.9932	4
dUrd	0.49	−11.27	0.9875	−2.67	2.01	0.9742	126.41	−32.37	20.9	0.9906	−11.35	−9.35	0.42	0.9880	−0.37	0.06	0.9952	4
dGuo	0.87	−13.5	0.9848	−2.97	2.46	0.9964	368.39	−83.45	4.92	0.9987	71.76	−25.60	1.33	0.9975	−0.98	0.13	0.9878	4
dThd	0.88	−11.48	0.9940	−2.38	2.09	0.9963	377.08	−89.26	5.63	0.9990	40.24	−18.27	1.14	0.9996	−1.02	0.16	0.9962	4
dAdo	1.31	−13.63	0.9916	−2.57	2.49	0.9973	979.15	−223.30	13.25	0.9980	56.69	−23.19	1.68	0.9995	−2.66	0.36	0.9894	4
Nitrobenzene	1.86	−3.12	0.9973	−0.36	2.36	0.9927	197.90	−188.50	47.24	0.9947	1.80	−4.38	2.06	0.9998	−9.20	5.33	0.9943	7
2,6-Dinitrobenzene	1.84	−2.84	0.9978	−0.43	2.99	0.9941	575.30	−524.20	121.61	0.9982	1.63	−0.05	2.06	0.9996	−8.91	5.64	0.9956	7
Benzene	2.38	−3.95	0.9966	−0.26	2.36	0.9957	159.40	−163.09	44.56	0.9886	2.63	−5.79	2.67	0.9999	−25.94	13.00	0.9782	7
2-Nitrotoluene	2.32	−3.75	0.9949	−0.35	2.85	0.9957	547.78	−497.41	116.08	0.9878	3.04	−5.88	2.66	0.9998	−24.27	12.41	0.9787	7
4-Nitrotoluene	2.33	−3.74	0.9953	−0.33	2.84	0.9955	564.05	−512.75	119.83	0.9876	2.92	−5.79	2.66	0.9999	−25.06	12.83	0.9791	7
3-Nitrotoluene	2.35	−3.71	0.9971	−0.29	2.81	0.9913	531.11	−492.74	117.92	0.9948	2.04	−5.14	2.58	0.9994	−24.93	13.02	0.9881	7
Toluene	2.35	−3.5	0.9980	−0.13	2.64	0.9896	538.17	−508.29	124.72	0.9962	1.50	−4.55	2.52	0.9994	−25.91	14.07	0.9926	7
2-Nitro-1,3-xylene	2.79	−4.35	0.9938	−0.30	3.30	0.9969	1445.20	−1275.47	284.93	0.9786	3.93	−7.10	3.23	0.9999	−61.35	29.18	0.9588	7
4-Nitro-1,3-xylene	2.85	−4.48	0.9926	−0.34	3.41	0.9975	1622.98	−1423.24	314.95	0.9763	4.44	−7.58	3.35	0.9999	−67.97	31.89	0.9530	7
m-Xylene	2.87	−4.20	0.9957	−0.11	3.19	0.9951	1736.00	−1551.07	352.34	0.9837	3.15	−6.41	3.23	0.9999	−75.94	36.87	0.9691	7
Hydrocortisone	2.16	−6.15	0.9955	−2.17	4.59	0.9882	181.86	−157.21	33.55	0.9286	3.10	−8.32	2.51	0.9981	−7.84	3.33	0.9204	5
Prednisone	2.21	−6.24	0.9970	−2.19	4.66	0.9904	191.14	−165.36	35.31	0.9307	2.72	−8.15	2.52	0.9989	−8.27	3.51	0.9221	5
Cortisone	2.29	−6.35	0.9976	−2.18	4.72	0.9832	208.38	−180.57	38.63	0.9308	1.22	−7.20	2.43	0.9979	−9.06	3.86	0.9239	5
Corticosterone	2.15	−5.37	0.9991	−1.97	4.87	0.9978	105.09	−100.31	24.20	0.9872	2.33	−7.25	2.52	0.9998	−4.92	2.47	0.9450	5
Cortexoloneone	2.03	−4.57	0.9947	−1.49	4.16	0.9998	135.36	−129.37	31.45	0.9872	5.46	−8.98	2.89	0.9998	−6.18	3.21	0.9462	5
Dexamethasone	2.17	−4.73	0.9943	−1.47	4.31	0.9998	175.46	−166.85	40.26	0.9863	5.89	−9.49	3.10	0.9999	−7.86	4.03	0.9408	5

relationship, Eq. (5), might be used to approximate the experimental data of k' as a function of F. The correlation coefficients (r^2) are always higher than 0.990, with the exception of the two cases, (see Tables 2 and 3). The log k' vs. log$(1/F)$ plots show the worst correlation. Eq. (2) approximates well for the experimental data of dGuo, dThd and dAdo only with the organic modifier of acetonitrile, where the correlation coefficients are more than 0.996. The two polynomial models, Eqs. (3) and (4), give relatively good correlation coefficients, but inherently, the parameters (C, D and E in Eq. (3), L, M and N in Eq. (4)) do not correlate with any properties of solutes or organic modifiers. The two equations are empirical equations only, and each has three parameters, one more parameter compared to the other three equations. However, Eq. (4) is especially useful when the content of organic modifier is higher (normally between 0.3 and 0.7 of F). The equation fits better because of logarithmic scale and more parameters to be fixed. The correlation coefficients of Eq. (5) are relatively low in the samples of nitro and steroid compounds. The data of the samples are obtained at higher content of organic modifier. In this case, the competitive adsorption of sample and organic modifier occurred on the C_{18} surface. This means that the Langmuir-type relationship, Eq. (5) is not adequate, and a more complex equation considering the interactions between sample and organic modifier is required. Finally, the slopes S of different solutes calculated by Eq. (1) for each organic modifier are approximately coincided from the two tables. The ratio of $S_{\text{acetonitrile}}/S_{\text{methanol}}$ for deoxyribonucleosides varies in a comparatively narrow range from 2.11 to 2.60 with an average value of 2.30, while the ratios of nitro compound are close to 1.00. In fact, these slopes are practically same for different deoxyribonucleosides. This conclusion for deoxyuridine and its derivatives was reported [12], where the slope values had not correlated to the hydrophobic properties of solutes. So the slopes of Eq. (1) characterize only the properties of organic modifier in the case of solute considered.

5. Conclusions

The retention factors of the deoxyribonucleosides with respect to the composition and type of mobile phase were measured under isocratic RP-HPLC conditions. From the results of comparison of the five retention models, Eqs. (1)–(5), the Langmuir-type retention model with the two parameters of A and B, Eq. (5), shows excellent agreements between the experimental retention factors and calculated values especially in the small content of organic modifier. This new model is established based on the Langmuir adsorption, so the parameters have the physical meaning. But it does not fit well in the large content of organic modifier. Over the whole range of organic modifier, Eq. (4) may be used as a better predictable approach than the simpler form of Snyder relation, Eq. (1).

6. Symbols

A, B:	empirical constants used in Eq. (5)
C, D, E:	empirical constants used in Eq. (3)
F:	volume fraction of organic modifier in mobile phase
k':	retention factor
k'_w:	retention factor for pure water as mobile phase
K, H:	empirical constants used in Eq. (2)
L, M, N:	empirical constants used in Eq. (4)
r^2:	regression coefficient defined by Eq. (6)
S:	empirical constants used in Eq. (1)
V_0:	retention volume of nonretained component
V_R:	retention volume of component

Acknowledgements

This work was financially supported by KOSEF (Grant No. 975-1100-001-2) and was performed in the High-Purity Separation Laboratory of Inha University (Inchon, South Korea). The author gratefully acknowledges the fruitful discussion with Dr. A.V. Larin of RAS.

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