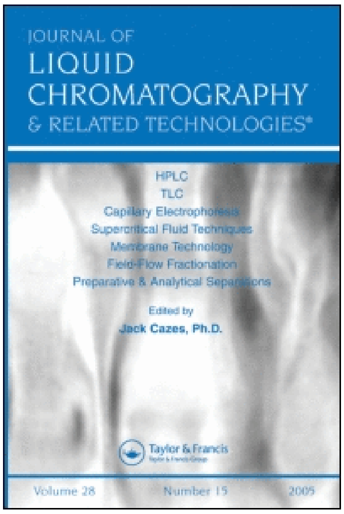

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REVERSED-PHASE HPLC RETENTION OF DEOXYRIBONUCLEOSIDES AS A FUNCTION OF MOBILE PHASE COMPOSITION

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ABSTRACT

Comparison of approaches describing retention of deoxynucleosides (dCyd, dUrd, dGuo, dThd, and dAdo) as a function of the mobile phase composition has been performed. The volume fraction (F) of organic modifier was changed from 0.05 to 0.30, and to 0.12 for methanol and acetonitrile, respectively. Linear regression has been carried out for the following equations: $\log k' = \log k'_w - S \times F$ (1), $\log k' = H + K\log(1/F)$ (2), $k' = A + B(1/F)$ (3), where k' is capacity factor, and $\log k'_w$, S, H, K, A and B are experimental coefficients. The best fits were obtained for the eqns. (1) and (3), and the poorest correlations were obtained for eqn. (2). The slope ratios $B_{methanol}/B_{acetonitrile}$ and $S_{acetonitrile}/S_{methanol}$ are changed in comparatively narrow ranges with average values of 2.86 and 2.30,

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respectively. Unlike the slope S of eqn. (1), the slope B of eqn. (3) can characterize the properties of solute and organic modifier simultaneously. For each solute, the intercepts A calculated for acetonitrile and methanol as organic modifiers are coincident closely . It is shown the validity of the coefficients of eqn. (3) to express the deoxyribonucleoside properties.

INTRODUCTION

Still now the theory of chromatographic retention of solutes under conditions of reversed-phase HPLC has not been worked out fully. An analysis of a number of approaches describing this phenomenon has been reported in the reviews [1,2]. It follows from the analysis, the the approaches developed have used some assumptions and can be applied only for qualitative predictions of solute chromatographic behaviour. Moreover, a majority of the relationships proposed has neither physical significance nor limited range of practical application.

For a given chromatographic column, the composition of the mobile phase determines the retention of solutes. The general approach assumes that reversed-phase retention obeys the linear relationship between log k′, where k′ is capacity factor, and volume fraction of organic modifier in the mobile phase [3]. It is really the true for a thermodynamically ideal binary solvent mixture . However, it was shown in [4] that methanol/water and acetonitrile/water mixtures are not ideal solutions. So the plots of log k′ vs. organic modifier fraction exhibit some curvature and are best fit by a quadratic equation [5], or they can be linearized by plotting vs. a spectroscopic function of the mobile phase: the so-called ET(30) index [1].

Nevertheless, even a formal description of solute chromatographic retention is very important and useful to systematize and to apply the experimental data. The capacity factor data vs. content of organic modifier in the mobile phase could be regarded as a measure of the hydrophobic character of the solutes of biological, environmental and agricultural interests. Moreover, the optimum conditions for the gradient runs could be established on the basis of the precise description of solute movement in chromatographic column.

The chromatographic behaviour of 5 deoxyribonucleosides have been studied in [6], and it has been shown the non-coincidence of the experimental and extrapolated values of capacity factors with a mobile phase of pure water. The purpose of this work is to compare the different approaches describing retention of deoxyribonucleosides with respect to composition of mobile phase.

METHODOLOGY

Prediction of retention under reversed-phase HPLC is based usually on some expected dependences of capacity factor k′ on mobile phase composition. Also, there are a number of approaches to the effect of the mobile phase on chromatographic retention. More often it is discussed in literature a problem of the extrapolation of experimental data to estimate the value of capacity factor for water as mobile phase (k'_w) [7]. The value of k'_w serves as a good descriptor and predictor of the solute hydrophobicity in biological systems [2]. In our analysis the results of data interpolations were used advantageously to estimate the same properties of a solute.

The retention under reversed-phase HPLC conditions obeys the linear relationship [3]:

$$\log k' = \log k'_w - S \times F, \qquad (1)$$

where k' refers to the solute capacity 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 and mobile phase composition.

Reversed phase slope and intercept values of eqn. (1) are regarded as a measure of the hydrophobic character of the solutes [8]. There are a number of papers reporting the use of eqn. (1) for the estimation of hydrophobic correlations. The peculiarities of eqn. (1) are discussed and reviewed elsewhere [1-8].

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. Kaibara and co-workers [9] defined the slope of $\log k'$ vs. $\log(1/F)$ as a good indicator for hydrophobicity. We used this dependence in the next form:

$$\log k' = K \log(1/F) + H, \qquad (2)$$

where K and H are empirical coefficients.

The novel relationship between the capacity factor and organic modifier content in the eluent was proposed in [10]. This relationship has used an assumption that organic modifier adsorption is described by Langmuir isotherm. The final equation can be expressed as follows:

$$k' \square A + B(1/F), \qquad (3)$$

where A and B are experimental coefficients, $A = k'_w\ h$, and $B = k'_w/b$, k'_w is the capacity factor in pure water, b is a constant of Langmuir equation characterizing the adsorption interaction between the organic modifier molecules and adsorbent surface, F is the organic modifier content in the mobile phase, and h is an empirical constant relating to the solute molecules and adsorbent surface interaction.

The eqn. (3) was obtained under a few of assumptions. But as it will be shown below, the deoxyribonucleoside chromatographic retention obeys the linear relationship between k′ and 1/F.

All equations were linearized by LOTUS 123™. The correlation coefficients are noted as r^2.

RESULTS AND DISCUSSION

A chromatographic retention of five deoxyribonucleosides (dCyd, dUrd, dGuo, dThd, and dAdo) with respect to the mobile phase composition has been studied under isocratic conditions of reversed-phase HPLC. The Waters column (30x0.39 cm) packed by 10 μm μ-Bondapak C18 was used. 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 elutions were performed by using an isocratic protocol at a flow rate of 1 ml/min. Methanol and acetonitrile were used as organic modifiers. The details of experimental part were described in [6].

In Figures 1-6, the experimental data and linear regression lines are shown for all approximations performed. With the exception of data of the eqn. (3), the slopes of plots obtained for different solutes remain fairly constant for each organic modifier, but the corresponding values of intercepts are changed strongly (Figures 1-4). Oppositely, the dependences of k′ vs. 1/F plots are characterized by the different magnitudes of slopes for each solute (Figures 5-6).

Linear regression has been carried out according to the eqns. (1-3) for each solute and organic modifier. The slopes, intercepts, and correlation coefficients calculated are summarized in Table 1. Clearly

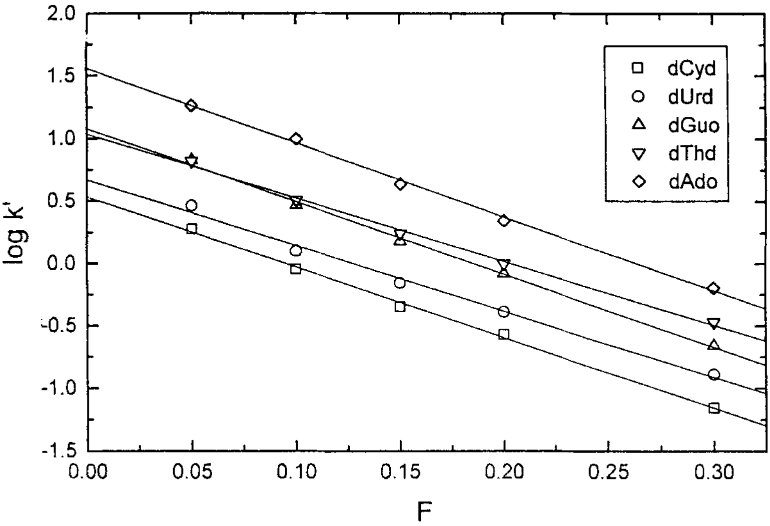

Figure 1. The plot of capacity factor vs. the methanol volume fraction approximated by using equation (1).

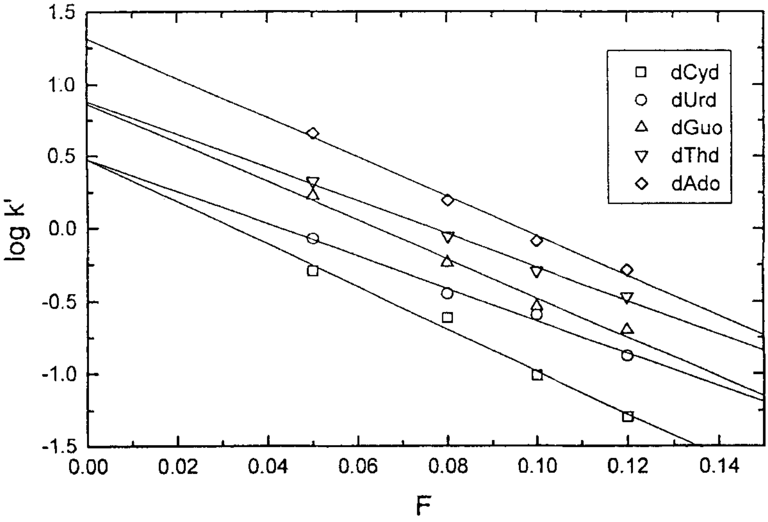

Figure 2. The plot of capacity factor vs. the acetonitrile volume fraction approximated by using equation (1).

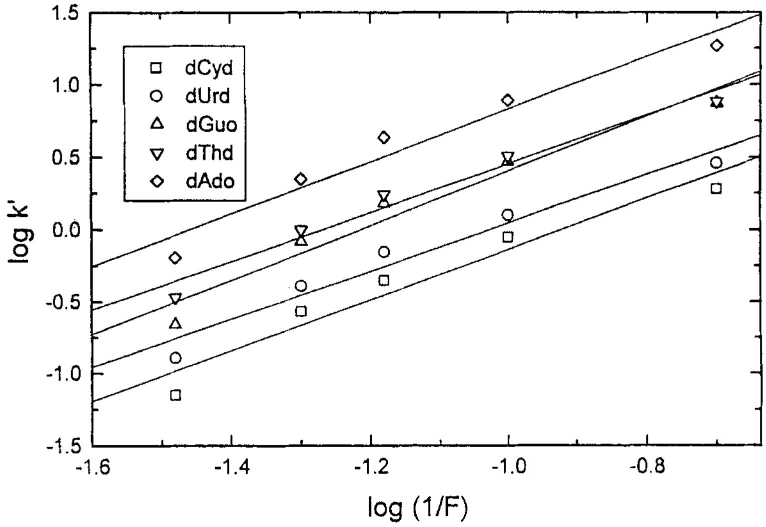

Figure 3. The plot of capacity factor vs. the methanol volume fraction approximated by using equation (2).

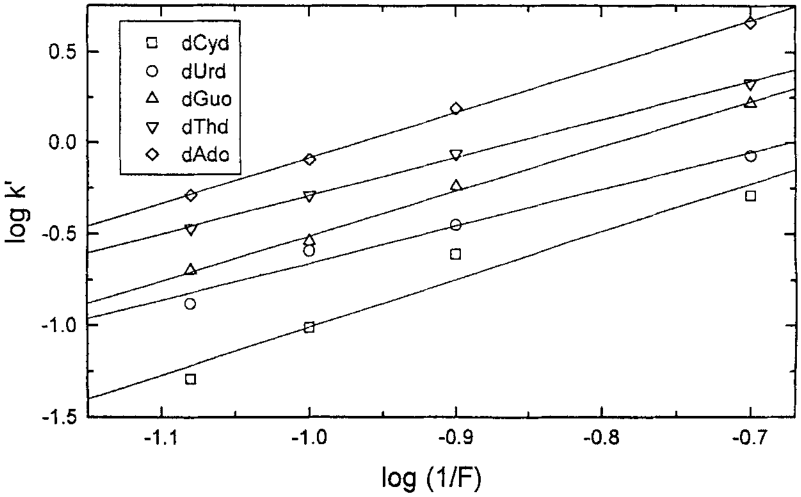

Figure 4. The plot of capacity factor vs. the acetonitrile volume fraction approximated by using equation (2).

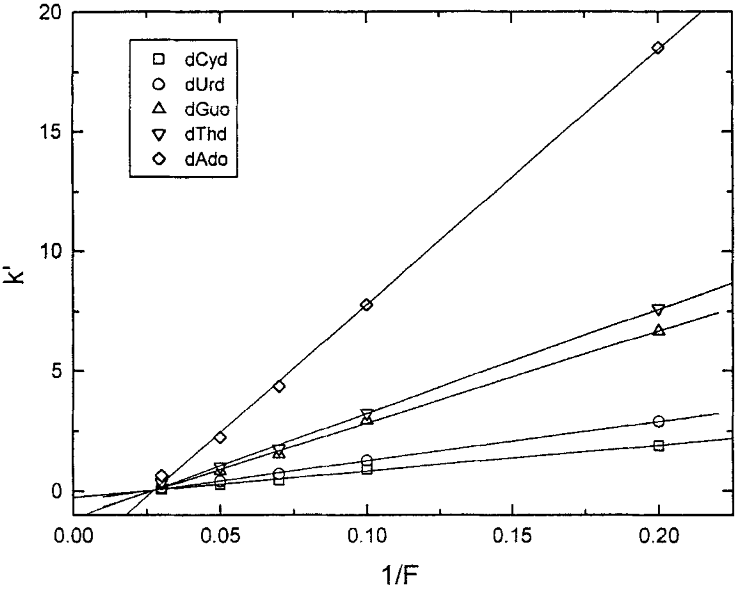

Figure 5. The plot of capacity factor vs. the methanol volume fraction approximated by using equation (3).

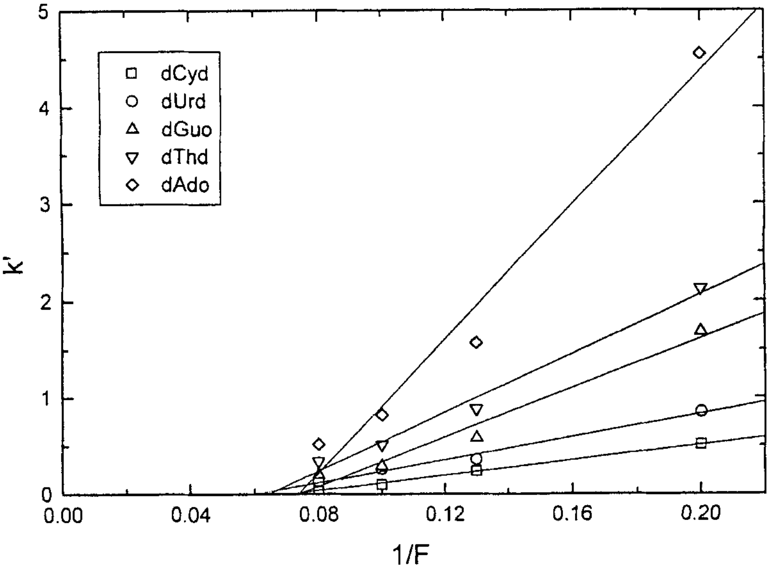

Figure 6. The plot of capacity factor vs. the acetonitrile volume fraction approximated by using equation (3).

TABLE 1
Slopes and Intercepts of Equations (1),(2), and (3)

Organic modifier	Solute	(1)			(2)			(3)		
		S	log k'_w	r^2	K	H	r^2	B	A	r^2
Methanol	dCyd	-0.06	0.53	0.998	1.77	1.63	0.938	10.92	-0.27	0.997
	dUrd	-0.05	0.67	0.993	1.67	1.72	0.960	16.67	-0.42	1.000*
	dGuo	-0.06	1.11	0.995	1.89	2.30	0.956	38.87	-1.06	0.999
	dThd	-0.05	1.08	0.990	1.69	2.14	0.968	44.03	-1.17	1.000*
	dAdo	-0.06	1.51	0.996	1.81	2.64	0.950	107.46	-2.96	1.000*
Acetonitrile	dCyd	-0.15	0.48	0.984	2.61	1.80	0.945	3.99	-0.28	0.991
	dUrd	-0.11	0.48	0.990	2.01	1.35	0.974	6.04	-0.37	0.996
	dGuo	-0.13	0.86	0.985	2.46	1.95	0.996	13.09	-0.98	0.988
	dThd	-0.11	0.88	0.994	2.09	1.80	0.996	15.56	-1.02	0.996
	dAdo	-0.14	1.32	0.992	2.49	2.41	0.997	35.64	-2.67	0.999

*1.000 means $r^2 \geq 0.9995$

the best fits are obtained when the eqns. (1) and (3) were used to approximate the experimental data k′ as a function of F. The correlation coefficients (r^2) are always higher than 0.990, with the exception of the three cases, where they are higher than 0.984 (see Table 1) .

The log k′ vs. log(1/F) plots have poorest correlations. The eqn. (2) approximates in a good manner the data obtained for dGuo, dThd, and dAdo only with acetonitrile in the mobile phase (the correlation coefficients are more than 0.996). But these slopes and intercepts do not correlate with any properties of solutes or organic modifiers. Thus, for further analysis we will consider only correlations obtained by using the eqns. (1) and (3).

The slopes S of different solutes calculated according to (1) for each organic modifier are approximately coincided. Their ratio $S_{acetonitrile}/S_{methanol}$ is varied in comparatively narrow range from 2.09 to 2.61 with an average value of 2.30 (Table 2). In fact, these slopes are practically the same for different deoxyribonucleosides. This conclusion for deoxyuridine and its derivatives was reported in [11], where the slope values had not correlated to the hydrophobic properties of solutes. So the slopes of eqn. (1) characterize only the properties of organic modifier in the case of solute considered. The property of the log k'_w intercept to characterize the hydrophobicity of deoxynucleosides was described in [6] in details.

The correlation coefficients of linear regression obtained by using of the eqn. (3) in the most cases are exceeded the ones obtained for eqn. (1). It is obvious that eqn.(3) describes the experimental data for deoxynucleosides not worse than eqn. (1).

For a given organic modifier, the slopes B and intercepts A are changed strongly with respect to different solutes (see Table 1). It

TABLE 2

Ratios of Slopes Calculated for Methanol and Acetonitrile According to Equations (1) and (3)

Solute	dCyd	dUrd	dGuo	dThd	dAdo	AVG.*
$S_{acetonitrile}/S_{methanol}$	2.61	2.09	2.23	2.17	2.40	2.30
$B_{methanol}/B_{acetonitrile}$	2.74	2.76	2.96	2.83	3.01	2.86

* Average value of slope ratios

indicates that the experimental coefficients A and B characterize the properties of solutes and they probably relate with the value of k'_w. This conclusion is confirmed by an analysis of slopes of eqn. (3). The slopes B are different for deoxyribonucleosides studied. Their ratios $B_{methanol}/B_{acetonitrile}$ is varied in comparatively narrow range from 2.74 to 3.01 with an average value of 2.86 (Table 2). So the ratios of slopes S and B can characterize the properties of organic modifier only. Unlike the slopes of eqn. (1), the slopes of eqn. (3) express the properties of solutes and organic modifier simultaneously.

As seen from the data of Table 1, the intercepts A calculated for acetonitrile and for methanol as organic modifiers are closely coincident. These results are illustrated in Figure 7 by straight line arising from origin. The same results were obtained previously by using of eqn. (1) in the region of the small content of organic modifier [6].

Thus, it is shown the validity of both the slope and the intercept of eqn. (3) to express the properties of deoxyribonucleosides. But the range of application of eqn. (3) is not defined fully. As it follows

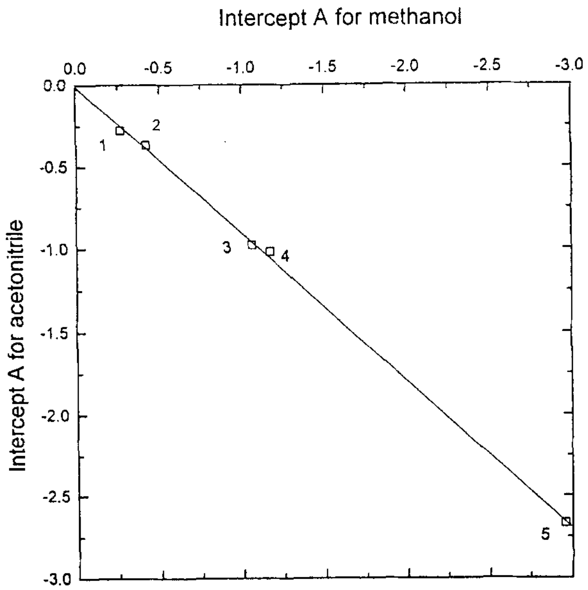

Figure 7. Comparison of intercepts A of equation (3) (deoxyribonucleosides: 1 = dCyd, 2 = dUrd, 3 = dGuo, 4 □ dThd, and 5 □ dAdo)

from an analysis of eqn. (3) [12], the features observed for deoxyribonucleosides are not the same for other substances. For example, the data of [7] have good correlations by using of equation (3), but the slopes of benzene solute calculated for different organic modifiers are equal.

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