

*Dedicated to the memory of
Professor Mircea D. Banciu (1941–2005)*

EXTRAPOLATIONS BY MULTIPLE REGRESSIONS OF THE RETENTION PARAMETERS FOR SEVERAL AROMATIC HYDROCARBONS IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

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Different elution conditions were applied in order to establish the dependence of the retention parameters on the nature of the organic modifier. The dependences of capacity factor for toluene, fluorene and fluoranthene on the organic modifier content (acetonitrile or methanol) were extrapolated for estimating their octanol/water partition coefficient. It was found out that the nature of the organic modifier influences but not significantly these extrapolated values. In order to correlate them with shake-flask experimental values and those established by fragment methodology several mathematical regressions were applied: linear or polynomial regression to fit the dependence between logarithm of capacity factor and organic modifier content in mobile phase, or high degree polynomial regression to fit the dependence of capacity factor and organic modifier content. The linear dependence between logarithm of experimental values of the capacity factor and logarithm of octanol/water partition coefficient of several aromatic hydrocarbons estimated by means of fragment methodology can be used in predicting the time retention of other unavailable hydrocarbons as standards.

INTRODUCTION

Retention mechanism(s) in reversed-phase liquid chromatography is still of actual debate.¹⁻³ The retention study of different compounds as depending on the experimental parameters (mobile phase composition, temperature, nature of hydrocarbonaceous chains from stationary phase, and so on) is the only possibility to have a view of the complex phenomena that take place during this process. Among these parameters, the content of the mobile phase may give a view on the ratio of forces acting on species during the elution process: solvation forces between analyte and the components from the mobile phase, on one hand, and hydrophobic forces between analyte and hydrocarbonaceous chains bound to the silica matrix from the stationary phase, on the other hand. Of course, the account of all forces involved into the process could be the only possibility to explain this process, but all the attempts so far have failed owing to the lack of any phenomenological model that comprises all forces. Adsorption model,⁴⁻⁶ partition model,⁷⁻⁹ solvophobic theory¹⁰ are only several trials to explain how the compounds are passing through a chromatographic column, and why are they spending a time interval higher than the dead time value. However, the lack of a general acceptable model for explaining the retention process in reversed-phase liquid chromatography has not influenced the possibility of interpreting in various ways the chromatographic data and exploiting them in analytical purposes.

The aim of this work is to study the behavior of several well-known hydrocarbons in reversed-phase liquid chromatography using different mobile phase compositions in order to extrapolate the resulted

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dependences to the theoretical case when the mobile phase is composed of only water. This situation is useful in estimating the distribution constant of analytes between octanol and water ($\log K_{o,w}$, or $\log P$) as one of the most important parameter describing the hydrophobicity of substances. The choice of these analytes is justified by the absence of any dissociable functional group that could be influenced during the retention process by the proton concentration from the mobile phase. For pure analytical applications the correlation between capacity factor and octanol/water partition coefficient is used in predicting the values of the retention time for other aromatic hydrocarbons that are not studied experimentally, but they are likely to occur in different studied samples.

EXPERIMENTAL

Experiments were performed with an Agilent 1100 liquid chromatograph, equipped with a degasser, quaternary pump, autosampler, column thermostat and diode-array detector (DAD). Chromatographic data were acquired by means of the Chemstation software (Agilent Technologies, Waldbronn, Germany). Detection was achieved at 254 ± 2 nm (against 480 ± 10 nm as reference wavelength).

Isocratic elutions were performed for a flow-rate of 1 mL/min, and different mobile phase compositions composed from water and an organic modifier (methanol – MeOH, or acetonitrile – ACN). Zorbax Eclipse XDB-C8 (150 mm length x 4.6 mm i.d. x 5 μ m particle diameter) was used for the entire study. According to the manufacturer, the constructive data for this chromatographic column are following: volume of the mobile phase ($V_{m,p.}$) is 1.52 mL, and the volume of the stationary phase ($V_{s,p.}$) given by the bonded chains from silica matrix is approximately 0.12 mL.

Column temperature was set up to 25°C, and the injection volume was 5 μ L. A standard solution containing 5 μ g/mL uracil, 10 μ g/mL toluene, 25 μ g/mL fluorene and 40 μ g/mL fluoranthene in acetonitrile was used.

All solvents were HPLC grade from Merck (Darmstadt, Germany). Water for chromatography (minimum resistivity – 18 M Ω and maximum Total Organic Content – 30 ppb) was produced within the laboratory of LaborMed Pharma S.A. by means of a TKA Lab HP 6UV/UF instrument and used during experiments. Aromatic hydrocarbons used in this study were of analytical purity.

The retention time of uracil was used as t_0 (dead time) for the calculation of capacity factor, $k' = (t_r - t_0)/t_0$, with t_r as the retention time for the analyte.

RESULTS AND DISCUSSION

The knowledge of dependence of the capacity factor, $k' = f(C_s)$, on the concentration of organic solvent (modifier) in mobile phase (C_s) is useful for several reasons:

- Could give a view on the ratio between forces that take part to the retention process;
- Could be used in predicting the retention and separation between without too many experiments;
- Could be used in predicting the retention variation for low variation of the mobile phase composition in the view of studying the robustness of the chromatographic separation;
- Could be another choice of estimating the distribution constant and hydrophobicity of various analytes, by extrapolating the function $f(C_s)$ to the limits of C_s .

The function $f(C_s)$ can be derived from the theoretical models applied to the retention process.^{13,14} However, two relationships are known from practice to describe the dependence of the capacity factor (k') in RP mechanism on the organic solvent (modifier) content (C_s , as volume fraction for instance, i.e. $C_s \in [0; 1]$) in mobile phase:

$$k' = \sum_{i=0}^n \alpha_i C_s^i \quad (1)$$

$$\log k' = \sum_{j=0}^m \gamma_j C_s^j \quad (2)$$

The regression coefficients α_i ($i = 0, \dots, n$) or γ_j ($j = 0, \dots, m$) can be estimated from the experimental dependence of k' or $\log k'$ on C_s (\log taken as a ten-base logarithm). For a narrow interval of C_s , these dependences are reduced to a linear fit, but for a wide interval of C_s they become polynomial functions of degree indicated in sum index from the above equations (n , or m). Extrapolation parameters (as indicated in Fig. 1) for these dependences are following:

a) For $C_s = 0$, one obtains the capacity factor corresponding to a mobile phase composed of only water, denoted by $k'_{o,w}$. In this case, the equations (1) and (2) give the two extrapolated parameters, $k'_{o,w} = \alpha_0$ and $k'_{o,w} = 10^{\gamma_0}$, respectively. At its turn, the capacity factor is correlated with the octanol/water partition coefficient assigned to the analyte ($K_{o,w}$). In accordance to the above remarks, the retention experiments should be performed in concentration range as much closer to 0 as possible.

b) For $C_s = 1$, one obtains the sum of all regression parameters from the above dependences, i.e. $k'_{o,s} = \sum_{i=0}^n \alpha_i$, or $\log k'_{o,s} = \sum_{i=0}^m \gamma_i$, respectively.

c) For $k' = 1$, or $\log k' = 0$, one obtains the values of C_s for which $t_r = 2 \cdot t_0$. This is a less discussed situation, which offers a view on the solubility ratio of analyte between octanol and organic solvent used in LC process.

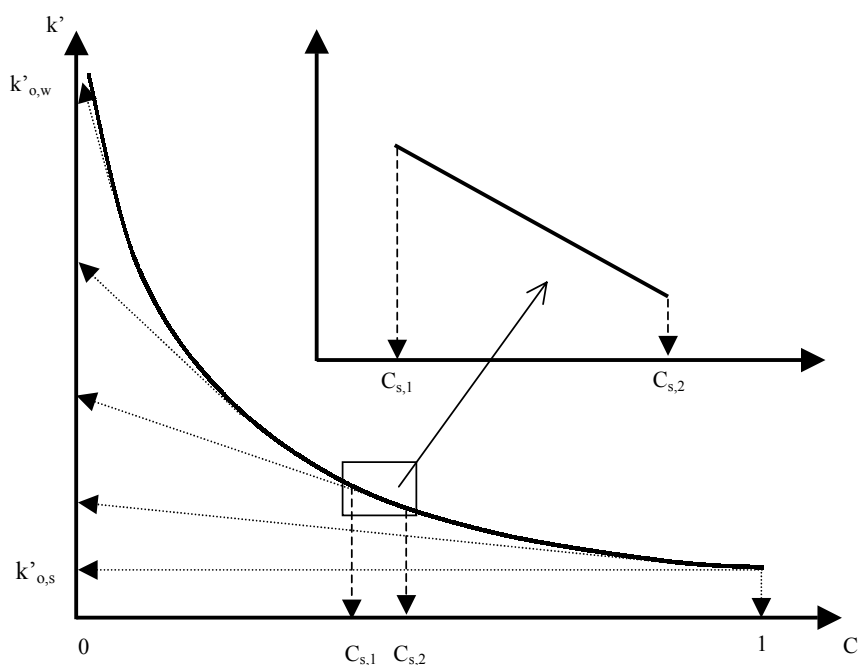


Fig. 1 – Graphical representation of the dependence between k' and C_s (as volume fraction), and extrapolations viewed for different concentration intervals used for the organic modifier from the mobile phase.

In this work, the capacity factors for toluene, fluorene and fluoranthene were studied using two organic modifiers: methanol (MeOH) and acetonitrile (ACN). The retention time values were measured for five concentrations for acetonitrile or methanol, within following intervals: [0.65; 0.75] (v/v) for acetonitrile as organic modifier, and [0.7; 0.8] (v/v) for methanol. A typical chromatogram for the mixture containing the three studied analytes and uracil as dead time indicator is given in Fig. 2.

The most common method to correlate the capacity factor with the organic modifier content in the mobile phase was to apply a simple linear regression of the type (2), i.e. with a degree $m = 1$. The plots for both organic modifiers are given in Fig. 3. The regression parameters obtained for dependences between $\log k'$ and C_s for the three studied analytes are given in the Tab. 1. They were calculated with very high correlation coefficients for the linear regression between $\log k'$ and C_s ($r^2 > 0.99$).

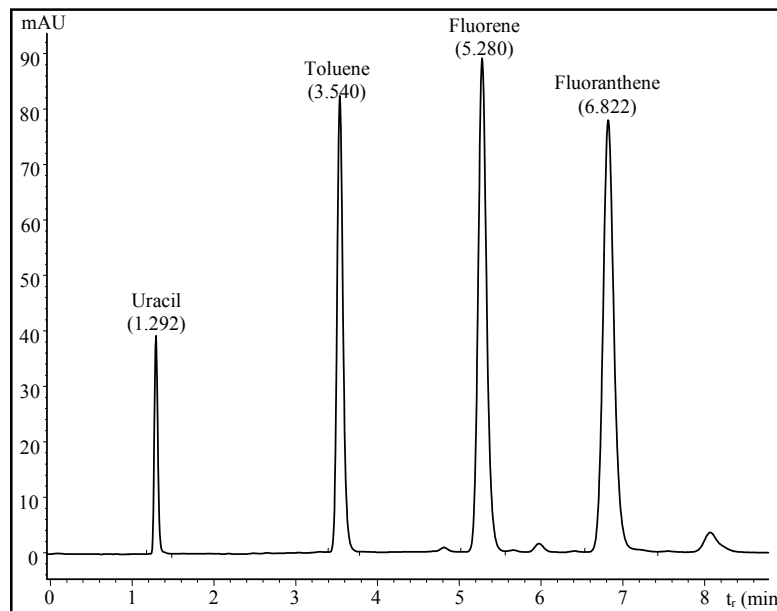


Fig. 2 – Chromatogram for a mixture containing 6 ppm uracil, 10 ppm toluene, 25 ppm fluorene and 40 ppm fluoranthene, using a mobile phase consisting of 32.5% water and 67.5% acetonitrile (v/v).

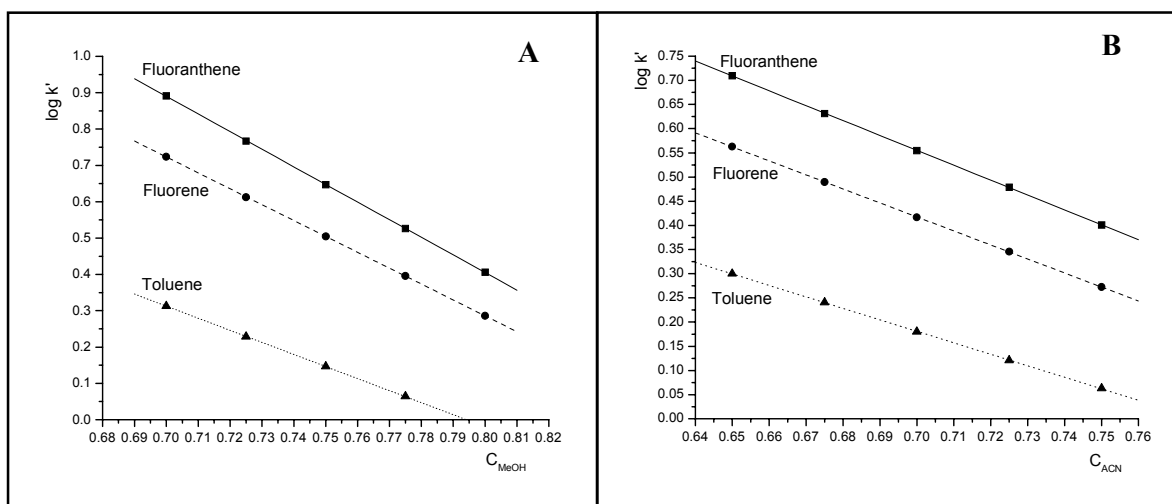


Fig. 3 – Dependences of capacity factor (as $\log k'$) on organic modifier content (C_s as volume fraction, $s = \text{MeOH}$, or ACN) in mobile phase for several aromatic hydrocarbons.

According to the previous discussions the parameter γ_0 can be used in estimating the partition constant for analytes ($\log K_{o,w}$), extrapolated for a mobile phase consisting of only water. The relationship between capacity factor and partition constant K of analyte between stationary phase and mobile phase is given by the equation:

$$\log K = \log k' + \log \frac{V_{m,p.}}{V_{s,p.}} \quad (3)$$

The second term in this equation is the most debatable in interpretation of the retention data for extra-analytical purposes.¹⁴ However, taking into consideration the data for $V_{m,p.}$ and $V_{s,p.}$ given in Experimental section, the second term from eq. (3) becomes 1.103. By means of this value one can estimate the partition constants for studied analytes, in different elution conditions and with the aid of the different mathematical models.

Table 1

Extrapolation parameters for the dependences of the retention according to the eq. (2).

Analyte	γ_0		γ_1	
	MeOH	ACN	MeOH	ACN
Toluene	2.637	1.843	-3.320	-2.375
Fluorene	3.784	2.446	-4.373	-2.898
Fluoranthene	4.283	2.711	-4.848	-3.080

Table 2

Octanol/water partition coefficients estimated from retention data using regression given by eq. (1), and compared to other theoretical and experimental approaches.

Analyte	log $K_{o,w}$		Retention log $K_{o,w}$ ***		Retention log $K_{o,w}$ ****	
	Theoretic*	Experimental**	MeOH	ACN	MeOH	ACN
Toluene	2.540	2.73	3.740	2.946	3.558	3.185
Fluorene	4.016	4.18	4.887	3.549	5.126	3.660
Fluoranthene	4.933	5.16	5.386	3.814	5.925	3.854

* By means of fragment methodology.¹⁵

** Shake-flask method.

*** Extrapolation from the eq. $\log k' = \alpha_0 + \alpha_1 C_s$.**** Extrapolation from the eq. $\log k' = \alpha_0 + \alpha_1 C_s + \alpha_2 C_s^2$.

As a general remark, in both situations $\log K_{o,w}$ obtained from retention experiments, using methanol or acetonitrile as organic modifier in mobile phase, is of same magnitude of order with experimental values, obtained by shake-flask methods, or with the theoretical values, obtained by the fragment methodology.¹⁵ The difference between $\log K_{o,w}$ predicted by the retention experiments and shake-flask methods decreases with the increase of the hydrophobicity of analyte. Unexpectedly, the differences between $\log K_{o,w}$ values estimated from retention experiments with methanol and with acetonitrile are rather significant, and explained by general behavior depicted in Fig. 1 only by different slopes of the curves $k' = f(C_s)$, as can be seen also from Tab. 2.

The attempt to correlate k' and C_s by means of a polynomial dependence according to eq. (1) was limited to the degree of 3, for higher than this value the extrapolation values α_0 being entirely in disagreement with the expected values. However, the use of polynomial regressions with degree higher than 1 is characterized by maximum and minimum values for k' , a fact that is entirely in disagreement with the experimental results. These extreme values can be calculated from the mathematical conditions ($dk'/dC_s = 0$). Thus, the maximum of k' is attained for the polynomial of degree 2 for:

$$C_s = -\frac{\alpha_1}{\alpha_2} \quad (4)$$

while, for a polynomial of degree 3 the extreme values are obtained for C_s given by the relationship:

$$C_s = \frac{-\alpha_2 \pm \sqrt{\alpha_2^2 - 4\alpha_1\alpha_3}}{2\alpha_3} \quad (5)$$

Taking into consideration that the interval for the values of C_s is [0; 1], these polynomial regressions could be used in the treatment of the retention data with the condition that the maximum and minimum points are attained for C_s values outer of the interval [0; 1]. The polynomial regression was applied also to the same retention data processed previously by logarithm dependence, and the regression parameters are summarized in Tab. 3.

For the three analytes the ratio $-\alpha_1/\alpha_2$ at which k' attains an extreme value is higher than 1, in both cases of the organic modifier. Therefore, the polynomial of degree 2 could be suitable to fit the retention data in these experiments. In case of the polynomial of degree 3, the parameters α_1 , α_2 and α_3 from the above table lead to impossible solutions of the eq. (5). Therefore, the domain [0; 1] for C_s is not characterized by extreme values for the dependence k' on C_s , in all situations.

As a remark, the extrapolated values of $\log K_{o,w}$ were higher for methanol than for acetonitrile, but in a less agreement with the experimental values, excepting toluene, than in case of using logarithmic dependence (eq. 2). Owing to its lower hydrophobicity, the retention curve $k' = f(C_s)$ for toluene is less variable on the parameter C_s than for the other studied solutes, and thus, the extrapolated value of this function approaches in a higher agreement to the real value $k'_{o,w}$ than for fluorene and fluoranthene.

Table 3

Octanol/water partition coefficients (as $\log K_{o,w}$) estimated from retention data using polynomial regressions given by eq. (2)

Organic solvent	Acetonitrile								
	Polynomial of degree 2				Polynomial of degree 3				
Analyte	α_0	α_1	α_2	$\log K_{o,w}$	α_0	α_1	α_2	α_3	$\log K_{o,w}$
Toluene	19.36	-42.61	24.44	2.390	22.37	-55.51	42.89	-8.79	2.453
Fluorene	44.69	-102.5	60.54	2.753	115.2	-405.4	493.9	-206.4	3.164
Fluoranthene	67.23	-155.9	92.85	2.931	203.1	-740.1	928.6	-398.0	3.411
Organic solvent	Methanol								
	Polynomial of degree 2				Polynomial of degree 3				
Analyte	α_0	α_1	α_2	$\log K_{o,w}$	α_0	α_1	α_2	α_3	$\log K_{o,w}$
Toluene	32.53	-72.09	40.77	2.615	103.6	-357.2	421.4	-169.2	3.118
Fluorene	123.9	-288.6	170.2	3.196	437.8	-1547.3	1850.5	-746.8	3.744
Fluoranthene	210.5	-497.7	297.2	3.426	800.6	-2864.1	3456.4	-1404.1	4.006

APPLICATION

The relationship (3) can be used in practice to derive an equation that correlates the hydrophobicity and the capacity factor in reversed-phase liquid chromatography. This equation is a linear dependence as following:

$$\log k' = \chi \log K_{o,w} + \theta \quad (6)$$

Here, the regression parameters χ and θ are obtained from studying the dependence between experimental values of $\log k'$ for several compounds and their theoretical values of $\log K_{o,w}$. In this way, an experimental parameter is correlated with a predicted parameters, such as $\log K_{o,w}$ estimated by means of the fragment methodology. If the retention is achieved with a mobile phase consisting in 30% water and 70% organic modifier (ACN or MeOH), the dependence (6) is characterized by the following parameters:

- i) For methanol: $\chi = 0.2454$; $\theta = -0.297$; correlation coefficient, $r = 0.9946$ ($t_0 = 1.463$ min);
- ii) For acetonitrile: $\chi = 0.1571$; $\theta = -0.217$; correlation coefficient, $r = 0.9998$ ($t_0 = 1.298$ min).

By means of these dependences and using the values of dead time (t_0) for each organic solvent used in LC retention, one can compute the estimated values for the capacity factors for the rest of other 14 polyaromatic hydrocarbons, as considered of interest in their monitorization from environmental samples. The retention time values of all 16 PAHs according to the previous mathematical model are given in Tab. 4. Comparison between predicted and experimental values for fluorene and fluoranthene shows that they are in a very good agreement.

Table 4

The expected value of the retention time (t_r) for the 16 PAHs in case of a mobile phase containing 70% organic modifier (methanol or acetonitrile) and 30% water, and C8 stationary phase, indicated in Experimental section; [experimental t_r (min) for MeOH: 9.21 – fluorene; 12.86– fluoranthene; for ACN: 4.69 – fluorene; 5.96 - fluoranthene]

#	Hydrocarbon	CAS	$\log K_{o,w}$ (theoretic)	$\log K_{o,w}$ (experimental)	Predicted retention time (t_r – min)	
					MeOH	ACN
1	Naphthalene	91-20-3	3.17	3.30	5.88	3.78
2	Acenaphthylene	208-96-8	3.94	3.94	8.29	4.57
3	Acenaphthene	83-32-9	4.02	3.92	8.60	4.83
4	Fluorene	86-73-7	4.15	4.18	9.15	4.67

Table 4 (continues)

Table 4 (continued)

5	Phenanthrene	85-01-8	4.34	4.35	10.02	5.08
6	Anthracene	120-12-7	4.34	4.45	10.02	5.08
7	Fluoranthene	206-44-0	4.93	5.16	13.40	5.98
8	Pyrene	129-00-0	4.93	4.88	13.40	5.98
9	Benz(a)anthracene	56-55-3	5.52	5.76	18.13	7.10
10	Chrysene	218-01-9	5.52	5.81	18.13	7.10
11	Benz(a)fluoranthene	205-99-2	6.11	6.11	24.72	8.48
12	Benz(k)fluoranthene	207-08-9	6.11	6.11	24.72	8.48
13	Benz(a)pyrene	50-32-8	6.11	6.75	24.72	8.48
14	Dibenz(a,h)anthracene	53-70-3	6.70	6.75	33.93	10.19
15	Benz(g,h,i)perylene	191-24-2	6.70	6.63	33.93	10.19
16	Indeno(1,2,3-c,d)pyrene	193-39-5	6.70	?	33.93	10.19

As can be observed, according to the correlation between $\log k'$ and $\log K_{o,w}$, several pairs of hydrocarbons are not separated in reversed-phase LC owing to the fact that they have the same theoretical $\log K_{o,w}$. However, in practice, using a mobile phase with starting low content in organic modifier and working in gradient elution in order to obtain reasonable retention time values, all these PAHs can be separated,¹⁶ although this mathematical approach predicts the same situation of not separating them. The only explanation is their solubility difference in mobile phase, a parameter that was not taken into account in the present study.

CONCLUSIONS

Experiments showed that the capacity factor in reversed-phase liquid chromatography was correlated with the composition of the mobile phase, regardless of the organic modifier used during the elution process. Their correlation can be achieved by different mathematical regressions, which does not differ significantly in what was the aim of this paper – their extrapolation for theoretical situation when the mobile phase is only water. This possibility is used in estimating the hydrophobicity of analytes, as given by the octanol/water partition coefficient. The agreement between values estimated from retention and those values estimated by means of fragment methodology or from classic experiments is fair. This model can be used in practice for predicting the retention time values for analytes by means of the correlation between capacity factor (as logarithm) and octanol/water partition coefficient (as logarithm).

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