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THE INFLUENCE OF COLUMN TEMPERATURE ON THE EXTRAPOLATED VALUES OF THE RETENTION FACTOR IN REVERSED-PHASE LIQUID CHROMATOGRAPHY FOR WATER AS MOBILE PHASE

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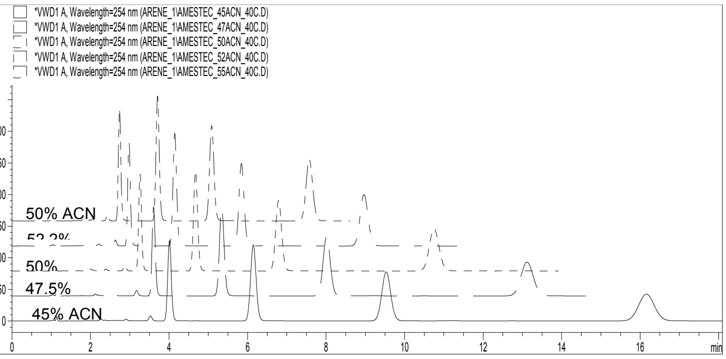
The dependences of the retention factor for some aromatic hydrocarbons at different column temperatures on the concentration of the organic modifier in mobile phase (acetonitrile) were studied under reversed-phase liquid chromatography, using two C18 chromatographic columns with the same constructive characteristics. From these dependences the extrapolated values of the retention factor for the aromatic hydrocarbons were calculated and then they were used for studying their temperature dependence by means of the van't Hoff plots. The graphs between the logarithm of the retention factor and the inverse value of absolute temperature of the column were characterized by good determination coefficients. The standard enthalpy variation associated to the transfer of studied hydrocarbons from the aqueous mobile phase to the stationary phase was calculated from these van't Hoff plots.



INTRODUCTION

In reversed-phase liquid chromatography (RP-LC) the dependence of the retention factor (k) on the concentration of the organic modifier ($C_{\text{o.m.}}$; %, volume/volume) in mobile phase is usually given by the empirical equation between decimal logarithm of k and $C_{\text{o.m.}}$:[1–3]

$$\log k = a + bC_{o.m.} \tag{1}$$

This dependence can be studied for any compound that has retentivity on C8, C18 or phenyl stationary phase for a certain domain of the organic modifier concentration.[4–7] Linear regressions based on the eq. 1 can be developed by using the set of experimental values (k, $C_{\text{o.m.}}$), and used in characterization of the retention behavior of the studied solutes on an used stationary phase.[8–10] This dependence is explained by the solvophobic theory and can be used in predicting the retention behaviour of non-polar or weakly polar organic compounds under RP mechanism.[11,12]

For $C_{\text{o.m.}} = 0$, the value of log k corresponds to the mobile phase consisting in water only, and usually this is designated as the extrapolated value of decimal logarithm of the retention factor, log k_w. From equation (1) it results that log $k_w = a$. On the

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other hand, the extrapolated value of the retention factor is correlated to the partition constant ($K_{w/s}$) of the solute distributed between the mobile phase (water) and the hydrophobic stationary phase, by the known formula:

$$k_w = \phi \cdot K_{w/s} \qquad (2)$$

A fundamental parameter[13,14] in eq. (2) is ϕ, which represents the phase ratio (the ratio between the volume of the stationary phase to the volume of mobile phase from the chromatographic column).

On the other hand, the dependence of the retention on temperature is described by the well-known van't Hoff equation, written as following:

$$\log k = -\frac{\Delta H^0}{2.303 \cdot R \cdot T} + \frac{\Delta S^0}{2.303 \cdot R} + \log \phi \qquad (3)$$

In eq. 3, ΔH^0 is the standard enthalpy for the transfer of the analyte from the mobile phase to the stationary phase, ΔS^0 is the standard entropy for the same partition process, R is the universal gas constant (8.314 J K^{-1} mol^{-1}), and T is the absolute column temperature (namely t + 273.15; t in °C). According to this equation the dependence between ln *k* and 1/T should be linear, unless some complex retention processes may occur that deviate this dependence from the linearity.[15–18]

As previously mentioned, an extrapolated value is calculated from the dependence of log *k* on $C_{\text{o.m.}}$, namely log k_w, which should be obeyed to the van't Hoff dependence. Such dependence is rarely reported by the literature, and it is the aim of this paper to investigate such dependences of extrapolated values of log k_w on 1/T for a series of common aromatic compounds. The thermodynamic parameters computed from these plots were compared to the usual data measured from retention with typical compositions of the mobile phase (mixtures of acetonitrile and water) used in reversed-phase separations and reported by the literature.

EXPERIMENTAL

This study was performed on an Agilent 1100 Series liquid chromatograph (Agilent Technologies) in the following configuration: degasser (G1379A), binary pump (G1312A), auto sampler (G1313A), column thermostat (G1316A), and diode array detector (G1315A). Chromatographic data were acquired by means of Agilent Chemstation software rev. B.01.03.

All solvents were HPLC (gradient) grade. Acetonitrile was purchased from Sigma-Aldrich (Germany). Water (resistivity minimum 18.2 MΩ and TOC maximum 30 ppb) was produced within the laboratory with a TKA Lab HP 6UV/UF instrument.

A standard solution containing 125 μg/mL of each aromatic hydrocarbons (benzene, toluene, ethylbenzene and propylbenzene) in acetonitrile was prepared, from which 1 μL was injected into the chromatographic column.

Separations were carried out using two different columns containing octadecylsilyl chemically bonded to silicagel (generally denoted by C18) as stationary phase. These columns have the following characteristics:

i) Hypersil BDS C18 (produced by Thermo Scientific): 100 mm length, 4.6 mm internal diameter, 5 μm particle size,

ii) Gemini 5u C18 (produced by Phenomenex): 100 mm length, 4.6 mm internal diameter, 5 μm particle size.

Mobile phase consisting in acetonitrile (ACN) and water was used in this study with the following compositions: 45; 47.5; 50; 52.5 and 55% ACN. Column temperatures were set-up at the following constant temperature: 25; 30; 35; 40; 45; and 50°C.

The value of retention factor *k* was calculated for each studied aromatic hydrocarbon with the formula $k = (t_r - t_0)/t_0$, where the t_r represents the retention time for the hydrocarbon, and t_0 represents the dead time of the separation, which was measured separately from the retention time of uracil under the same chromatographic conditions applied to the studied hydrocarbons (mobile phase composition and temperature).

RESULTS AND DISCUSSION

Retention factor (*k*) for the four aromatic hydrocarbons was measured using various compositions of the mobile phase mentioned in Experimental section, for different set-up values of the column temperature. For example, some typical separations of the four aromatic hydrocarbons are illustrated in Fig. 1, using specified mobile phase compositions and a constant column temperature.

Linear regressions applied to the dependences log *k* versus $C_{\text{o.m.}}$ (eq. 1) lead to the regression parameters *a* and *b* given in Table 1, for the six temperatures used in this study. All regressions were characterized by high determination coefficients ($r^2 > 0.9900$). The extrapolated values of log *k* for mobile phase consisting in water only, namely log k_w, depend on the temperature of the column. Theoretically, this parameter is expected to be related to the temperature T, according to eq. 3. These dependences are shown in Fig. 2, and they are characterized by very high determination coefficients, and thus it can be concluded that the extrapolated values log k_w for the studied hydrocarbons are obeyed to the van't Hoff rule.

A similar study was undertaken for another C18 chromatographic column, indicated in Experimental section by Gemini 5u C18. The results for the dependence between log *k* and $C_{\text{o.m.}}$ are listed in Table 2. As can be seen, the differences between the regression parameters obtained for the two chromatographic columns are very small. However, when the value of the intercept (log k_w) is represented against 1/T, this time the linear dependences are characterized by smaller determination coefficients ($r^2 > 0.9000$), as can be seen from Fig. 3.

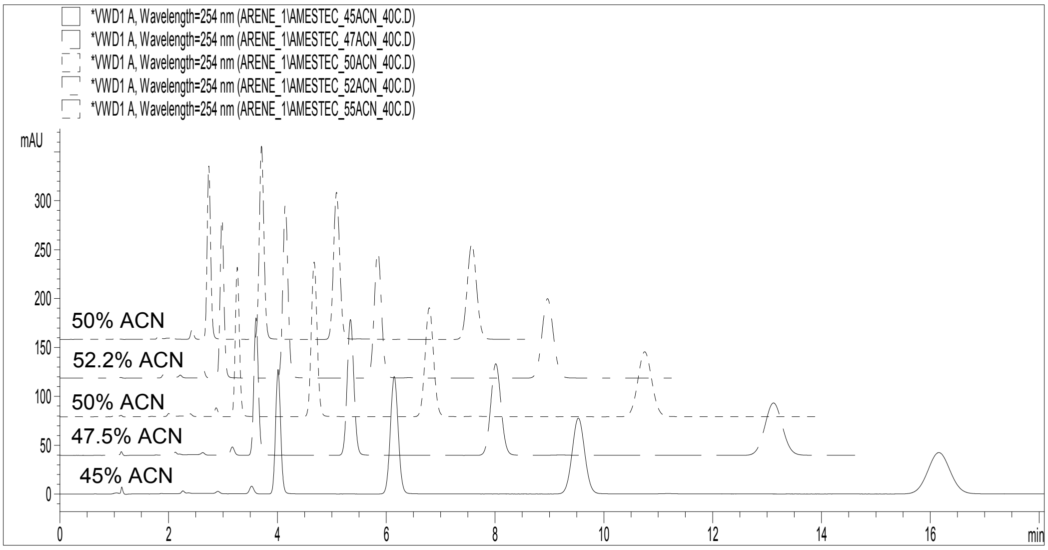

Fig. 1 – Overlaid chromatograms obtained for separation of the four aromatic hydrocarbons on Hypersil C18 column at different mobile phase compositions (45 – 55% acetonitrile, v/v), at 40°C.

Table 1

Regression parameters calculated for the linear dependences given by eq. 1 for the four studied hydrocarbons, at indicated column temperatures (Column: Hypersil C18 BDS; organic modifier: acetonitrile)

t (^{o}C)	**a = k_w**	**b**	**r^2**	**a = k_w**	**b**	**r^2**
	Benzene			**Toluene**		
25	1.7870	-0.0276	0.9989	2.1992	-0.0315	0.9988
30	1.7287	-0.0268	0.9989	2.13916	-0.0307	0.9989
35	1.6596	-0.0261	0.9991	2.0638	-0.0298	0.9992
40	1.5866	-0.0251	0.9993	1.9954	-0.0289	0.9992
45	1.5408	-0.0246	0.9994	1.9438	-0.0284	0.9992
50	1.4886	-0.0241	0.9996	1.8832	-0.0277	0.9994
	Ehtylbenzene			Propylbenzene		
25	2.6066	-0.0356	0.9986	3.0632	-0.0400	0.9983
30	2.5451	-0.0348	0.9988	2.9952	-0.0391	0.9987
35	2.4586	-0.0337	0.9991	2.8996	-0.0379	0.9989
40	2.3956	-0.0329	0.9991	2.8300	-0.0371	0.9988
45	2.3382	-0.0323	0.9992	2.7732	-0.0365	0.9989
50	2.2772	-0.0317	0.9992	2.7036	-0.0358	0.9989

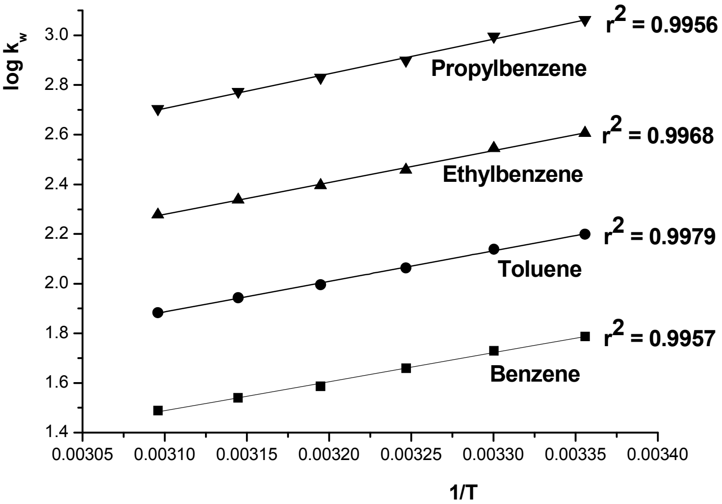

Fig. 2 – Dependence of the extrapolated values of k_w on $1/T$ for the studied aromatic hydrocarbons (Hypersil BDS C18 column).

Table 2

Regression parameters calculated for the linear dependences given by eq. 1 for the four studied hydrocarbons, at indicated column temperatures (Column: Gemini 5u C18; organic modifier: acetonitrile)

t (°C)	a = k_w	b	r^2	a = k_w	b	r^2
	Benzene			**Toluene**		
25	1.770	-0.0253	0.9998	2.176	-0.0294	0.9996
30	1.743	-0.0252	0.9997	2.146	-0.0293	0.9996
35	1.684	-0.0247	0.9996	2.082	-0.0287	0.9993
40	1.664	-0.0247	0.9995	2.072	-0.0289	0.9996
45	1.582	-0.0236	0.9996	1.972	-0.0275	0.9995
50	1.552	-0.0235	0.9989	1.943	-0.0274	0.9991
	Ehtylbenzene			Propylbenzene		
25	2.580	-0.0336	0.9994	3.021	-0.0380	0.9989
30	2.552	-0.0336	0.9991	2.995	-0.0380	0.9988
35	2.478	-0.0328	0.9992	2.915	-0.0372	0.9987
40	2.478	-0.0332	0.9997	2.917	-0.0376	0.9996
45	2.368	-0.0316	0.9995	2.796	-0.0359	0.9993
50	2.335	-0.0315	0.9991	2.759	-0.0357	0.9990

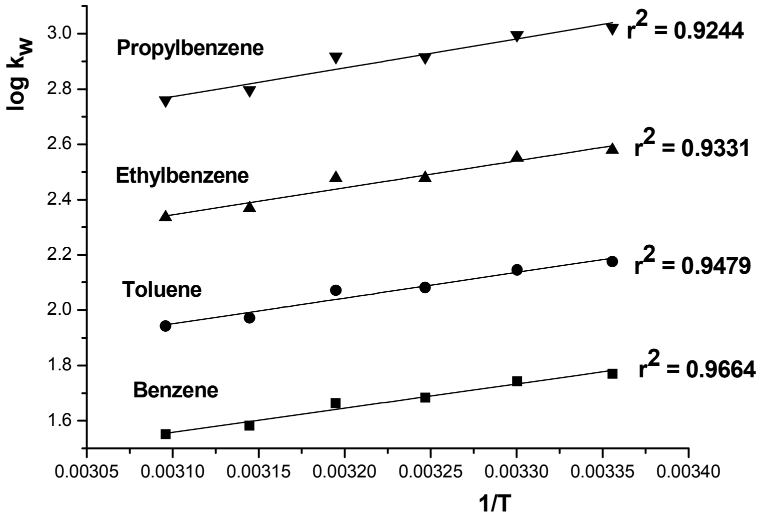

Fig. 3 – Dependence of the extrapolated values of k_w on $1/T$ for the studied aromatic hydrocarbons (Gemini 5u C18 column).

In both cases a linear van't Hoff plot was obtained for the dependence between log k_w and 1/T (with regression parameters denoted this time by *a*' and *b*'). Using the eq. 3, the value of ΔH^0 can be calculated corresponding to an hypothetical partition of the studied hydrocarbons between water as mobile phase and C18 stationary phase. The value of ΔH^0 can be calculated from the slope of the van't Hoff plots, *i.e.* $\Delta H^0 = -2.303 \times b' \times R$. These values for the two columns and the four aromatic hydrocarbons are listed in Table 3. These values are of the same magnitude with the values reported for other hydrophobic compounds, and for mobile phases consisting in different mixtures of organic modifier (acetonitrile) and water.[19–21] A conclusion that can be noticed from the values listed in Table 3 is referred to the hydrophobic character of the studied hydrocarbons: the more hydrophobic character of the studied compound, the higher value of the standard enthalpy for the compound transfer from mobile phase to the stationary phase.

Theoretically, the value of ΔS^0 could be calculated from the intercept of the linear regression, *a*', but it contains a term specific to the stationary phase, namely log ϕ (eq. 3). On the other hand, this parameter and the partition constant ($K_{w/s}$) from eq. 2 are both temperature dependent. Considering that the variation of ϕ with temperature is however small within the temperature interval used in this study, one can estimate the value of ΔS^0 from the intercept with formula: $\Delta S^0 = 2.303 \times R(a' - \log \phi)$. According to the literature, a fair approximation of a mean value of the phase ratio ϕ over the used temperature interval can be considered as 0.25.[22,23] With this value the formula of calculating the standard entropy variation becomes: $\Delta S^0 = 19.15 \times (a' + 0.602)$. The values resulted from these calculation are given in Table 3.

Table 3

The values for the standard enthalpy variation (ΔS^0) calculated from van't Hoff plots corresponding to the dependences of extrapolated retention factor on 1/T

Hydrocarbon	**Hypersil BDS C18 column**				**Gemini 5u C18 column**			
	b'	**ΔH^0 (kJ/mol)**	***a'***	**ΔS^0 (J/mol K)**	***b'***	**ΔH^0 (kJ/mol)**	***a'***	**ΔS^0 (J/mol K)**
Benzene	1172.1	-22.440	-2.145	-29.54	874.7	-16.747	-1.153	-10.55
Toluene	1229.8	-23.545	-1.926	-25.35	931.5	-17.835	-0.937	-6.41
Ethylbenzene	1282.9	-24.564	-1.698	-20.98	975.1	-18.670	-0.677	-1.43
Propylbenzene	1394.9	-26.707	-1.618	-19.45	1044.9	-20.006	-0.467	+2.58

According to the data from Table 3, the variation of the standard entropy with the hydrophobic character of the aromatic hydrocarbons is inverse to the variation of enthalpy. This could be correlated to the molecular dimensions, which could explain the decrease of ΔS^0 from benzene to propylbenzene. These variations of the standard entropy corresponding to the transfer of the analyte from water as mobile phase into the stationary phase are however much higher for Hypersil BDS column than those obtained for other hydrophobic compounds based on mixtures of acetonitrile and water as the mobile phase and using C18 stationary phase, when the reported interval of variations for ΔS^0 is [-20; -2 J/mol K].[24] For Gemini column the values of ΔS^0 are much smaller than in case of the previous column, with the remark that the calculated value of extrapolated ΔS^0 for propylbenzene becomes positive.

CONCLUSIONS

The extrapolated values of the retention factor for some aromatic hydrocarbons for mobile phase consisting in water only ($\log k_w$) can be calculated from the retention studies carried out at different constant column temperatures within the interval [20; 50°C]. The values of $\log k_w$ obtained for four aromatic hydrocarbons and two stationary phases are very well correlated with the absolute column temperature (1/T) according to the van't Hoff rule. The values of the variation of standard enthalpy (ΔH^0) can be calculated for each studied compound from van't Hoff plots, while for calculating the variation of the standard entropy (ΔS^0) an estimated value of the phase ratio of the used chromatographic column is required. The values of ΔH^0 and ΔS^0 calculated from extrapolated values of the retention factor ($\log k_w$), for water as mobile phase appear to be higher than the values obtained with mobile phases based on mixtures of acetonitrile and water.

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