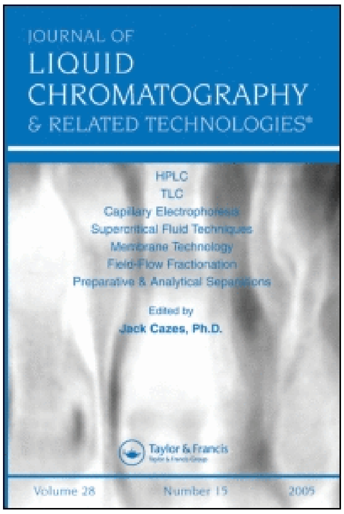

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MODELLING RETENTION IN REVERSED PHASE LIQUID CHROMATOGRAPHY IN RELATION TO TEMPERATURE AND SOLVENT COMPOSITION. APPLICATION TO THE SEPARATION OF SEVEN P-HYDROXYBENZOIC ESTERS

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ABSTRACT

The retention of seven closely related p-hydroxybenzoic esters in reversed phase liquid chromatography using a different volume fraction θ of methanol in the methanol-water mixture was studied with the determination of the separation factor α at different temperatures. Gibbs Helmholtz parameters $\Delta(\Delta H)$ and $\Delta(\Delta S)$ of two adjacent compounds on a chromatogram were obtained from $\ln\alpha$ versus $\frac{1}{T}$ plots. In this chromatographic system, as θ increases, $\Delta(\Delta H)$ and $\Delta(\Delta S)$ decrease and were found to vary quadratically with θ. A temperature dependent reversal of the elution order was studied and the mobile phase composition and column temperature were optimized to obtain the best separation conditions. A column temperature of 25 °C with a volume fraction $\theta = 0.52$ gave the best separation conditions.

INTRODUCTION

Retention in reversed phase liquid chromatography (RPLC) is still not fully understood and is a matter of debate in the chromatographic litterature.

The retention mechanism has been studied in RPLC by measuring thermodynamic parameters [1-9].

This paper, investigate over a wide range of volume fractions of methanol θ in the methanol/water mixture, the thermodynamic behavior of seven p-hydroxybenzoic esters. A temperature dependent reversal of the elution order and a method to separate these compounds were evaluated.

MATERIALS AND METHODS

Reagents

The chromatographed compounds were p-hydroxybenzoic esters. The straight chain esters, methyl to butyl (MeR, EtR, PrR, BuR) were purchased from Interchim (Montluçon, France). The branch chain esters isopropyl, isobutyl and secbutyl (IpR, IbuR, SbuR) were synthetized in our laboratory by an esterification reaction [10]. Methanol was analytical grade HPLC. All the compounds were diluted in methanol with a concentration of 10 mg/mL.

Apparatus

The HPLC system consisted of a HPLC Waters pump 6000 A (Saint-Quentin en Yvelines, France) an Interchim Rheodyne injection valve Model 7125 (Montluçon, France) fitted with a 20 μl sample loop and a Merck 4500 diode array detector (Nogent-sur-Marne, France). A Merck, RP 18 column (Lichrocart 50943, 119 mm x 4 mm I.D., 5 μm particle size) (Nogent-sur-Marne, France) was used with temperature controlled by an Interchim oven TM N°701 (Montluçon, France). Overall temperature control was maintained within ± 1°C with a variation from 26°C to 50°C. The mobile phase with a flow rate of 0.90 mL/min was a methanol water mixture with fraction of methanol θ varying from 0.4 to 0.8. Weaker percentages were not used because of the excessively high column pressure obtained with $\theta = 0.4$.

Methods

Thermodynamic relationships: valuable information can be gained by examining the temperature dependence of the separation factor α which is given by the equation:

$$\ln\alpha = -\frac{\Delta(\Delta H)}{RT} + \frac{\Delta(\Delta S)}{R} \quad (1)$$

where α is the separation factor between two compounds i and i+1 on the chromatogram

$$\alpha = \frac{k_{i+1}}{k_i} \quad (2)$$

k_{i+1} (respectively k_i) is the retention factor of compound i+1 (respectively i), $\Delta(\Delta H)$ (respectively $\Delta(\Delta S)$) the difference in the dissolution enthalpy (respectively dissolution entropy) between two compounds i and i+1, R the gas constant and T the column temperature. These differences in the dissolution enthalpy $\Delta(\Delta H)$ and entropy

$\Delta(\Delta S)$ are respectively a measure of polar and configuration effects. The difference in the dissolution free energy $\Delta(\Delta G)$ is given by:

$$\Delta(\Delta G) = \Delta(\Delta H) - T\,\Delta(\Delta S) \qquad (3)$$

RESULTS AND DISCUSSION

The experimental α values were calculated from the chromatograms for each adjacent compound pair noted (i, i+1). The results were processed by a computer and $\ln\alpha$ versus $\frac{1}{T}$ were plotted. The correlation coefficients were higher than 0.991. The typical standard deviation of the intercept and slope were respectively 0.005 and 0.03. Since these data were linear it was possible to calculate $\Delta(\Delta H)$ and $\Delta(\Delta S)$ for this system. Table 1 contains a complete list of the $\Delta(\Delta H)$ and $\Delta(\Delta S)$ values obtained for all solutes with different volume fractions of methanol θ in the methanol-water mixture. From these experimental data, it would appear that $\Delta(\Delta H)$ and $\Delta(\Delta S)$ vary quadrically with θ :

$$\Delta(\Delta H) = \epsilon_1\,\theta^2 + \beta_1\,\theta + \gamma_1 \qquad (4)$$

$$\Delta(\Delta S) = \epsilon_2\,\theta^2 + \beta_2\,\theta + \gamma_2 \qquad (5)$$

Combining equations 1, 4, 5 the following was obtained:

$$RT\ln\alpha = \epsilon_1\,(-\theta^2) + \beta_1\,(-\theta) + \gamma_1\,(-1) + \epsilon_2\,(T\theta^2) + \beta_2\,(T\theta) + \gamma_2\,(T) \qquad (6)$$

where α is the separation factor between two compounds for a couple (θ, T).

The values of ϵ_1, β_1, γ_1 ϵ_2, β_2, γ_2, were determined from a matrix product:

$$\underline{RT\ln\alpha} = M.\,\underline{p} \qquad (7)$$

where

$$RT\ln\alpha = \begin{vmatrix} RT\ln\alpha_1 \\ RT\ln\alpha_2 \\ \cdot \\ \cdot \\ \cdot \\ \cdot \\ \cdot \\ \cdot \\ RT\ln\alpha_n \end{vmatrix} \qquad P = \begin{vmatrix} \epsilon_1 \\ \beta_1 \\ \gamma_1 \\ \epsilon_2 \\ \beta_2 \\ \gamma_2 \end{vmatrix}$$

$$M = \begin{vmatrix} -\theta_1^2 & -\theta_1 & -1 & T\theta_1^2 & T\theta_1 & T \\ -\theta_2^2 & -\theta_2 & -1 & T\theta_2^2 & T\theta_2 & T \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ -\theta_i^2 & -\theta_i & -1 & T\theta_i^2 & T\theta_i & T \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ -\theta_n^2 & -\theta_n & -1 & T\theta_n^2 & T\theta_n & T \end{vmatrix}$$

where α_1, $\alpha_2 \ldots \alpha_n$ are the experimental values of the α recorded for each pair of temperature and volume fraction of methanol (T, θ).

$\underline{P}$ was calculated as:

$$\underline{P} = (M^tM)^{-1}\,(M^t\,\underline{RT\ln\alpha}) \qquad (8)$$

where M^t is the transposed matrix of M. The coefficients α_1, β_1, γ_1 and α_2, β_2, γ_2 are given in Table 2. The coefficient determination for these fits was always superior or

Table 1: Values of Δ(ΔH) (kJ/mol), Δ(ΔS) (J/mol/K) for different mobile phase compositions

	Volume Fraction θ of methanol							
	0.50		0.55		0.60		0.65	
peak pair[a]	Δ(ΔH)	Δ(ΔS)	Δ(ΔH)	Δ(ΔS)	Δ(ΔH)	Δ(ΔS)	Δ(ΔH)	Δ(ΔS)
(1,2)	-1.40	+6.63	-1.48	+6.08	-1.59	+5.60	-1.60	+5.30
(2,3)	-5.95	-2.56	-6.86	-6.61	-7.66	-10.17	-8.37	-13.21
(3,4)	-7.50	-9.44	-8.29	-13.55	-8.88	-17.52	-9.64	-20.53
(4,5)	-3.21	-6.77	-3.48	-7.89	-3.72	-8.99	-3.93	-9.79
(5,6)	-4.77	-10.96	-5.26	-13.27	-5.71	-15.40	-6.11	-17.34
(6,7)	-8.17	-24.12	-8.96	-27.73	-9.79	-30.86	-10.43	-33.55

Volume Fraction θ of methanol						
	0.70		0.75		0.80	
peak pair[a]	Δ(ΔH)	Δ(ΔS)	Δ(ΔH)	Δ(ΔS)	Δ(ΔH)	Δ(ΔS)
(1,2)	-1.78	+4.80	-1.82	+4.40	-1.91	4.24
(2,3)	-8.97	-15.77	-9.48	-17.82	-9.87	-19.37
(3,4)	-10.20	-23.46	-10.99	-26.00	-11.09	-29.94
(4,5)	-4.11	-10.57	-4.26	-11.24	-4.39	-11.79
(5,6)	-6.47	-19.09	-6.77	-20.66	-7.04	-22.04
(6,7)	-11.03	-35.79	-11.51	-37.59	-11.89	-39.95

[a]: N° in peak pairs correspond to :
1 Methyl p-hydroxybenzoic ester
2 Ethyl p-hydroxybenzoic ester
3 Isopropyl p-hydroxybenzoic ester
4 Propyl p-hydroxybenzoic ester
5 Secbutyl p-hydroxybenzoic ester
6 Isobutyl p-hydroxybenzoic ester
7 Butyl p-hydroxybenzoic ester

Table 2: Values of polynomial coefficients (equations 4 and 5) for $\Delta(\Delta H)$ and $\Delta(\Delta S)$

	$\Delta(\Delta H)$			$\Delta(\Delta S)$		
Compound pair[a]	α_1	β_1	γ_1	α_2	β_2	γ_2
(1,2)	2700	-5400	675	11.36	-22.72	15.15
(2,3)	20234	-39412	8700	99.92	-185.94	65.43
(3,4)	15000	-31500	4500	75.00	-160.00	52.32
(4,5)	5500	-11000	900	22.36	-45.82	10.54
(5,6)	9205	-19544	2700	37.34	-85.48	22.44
(6,7)	19161	-37312	5698	88.92	-164.94	36.09

[a]: see Table 1

equal to 0.995. Table 3 shows experimental and calculated values for $\Delta(\Delta H)$ and $\Delta(\Delta S)$ with $\theta = 0.60$.

For all compound pairs, $\Delta(\Delta H)$, $\Delta(\Delta S)$ and the separation factor α, decreased when θ increased. This proves that the polar and configuration effects also decrease when solvent polarity decreases. Using equation 3, the particular temperature T at which $\Delta(\Delta G)$ is nil with no resolution between the two adjacent compounds was determined. This temperature is expressed by:

$$\bar{T} = \frac{\Delta(\Delta H)}{\Delta(\Delta S)} \quad (9)$$

Combining equations 4, 5, 9:

$$\bar{T} = \frac{\epsilon_1\theta^2 + \beta_1\theta + \gamma_1}{\epsilon_2\theta^2 + \beta_2\theta + \gamma_2} \quad (10)$$

For a temperature higher than $\bar{T}$, the two compound elution orders should have been reversed. In our case, within the temperature range [25°C-50°C], the elution reversal was not detected. Therefore, whatever the factor variation, all the compounds were arranged on the chromatogram in the same order.

Nevertheless, the disappearance of resolution was observed at a temperature close to the calculated temperature $\bar{T} = 40°C$ for the peak pair (IbuR, BuR) with $\theta = 0.65$. When equation 6 is rewritten, the separation factor α relating to column temperature T and the volume fraction of methanol θ, is given by:

$$R\ln\alpha = \theta^2\left(\epsilon_2 - \frac{\epsilon_1}{T}\right) + \theta\left(\beta_2 - \frac{\beta_1}{T}\right) + \left(\gamma_2 - \frac{\gamma_1}{T}\right) \quad (11)$$

Table 3: Experimental and calculated values of $\Delta(\Delta H)$ (kJ/mol) and $\Delta(\Delta S)$ (J/mol/K) for $\theta = 0.60$

peak pairs[a]	$\Delta(\Delta H)_{exp}$	$\Delta(\Delta H)_{cal}$	e[b]	$\Delta(\Delta S)_{exp}$	$\Delta(\Delta S)_{cal}$	e (%)
(1.2)	-1.67	-1.59	4.79	+5.38	+5.60	3.92
(2.3)	-7.44	-7.66	2.87	-9.75	-10.16	4.04
(3.4)	-8.56	-9.00	4.88	-16.90	-17.23	1.92
(4.5)	-3.64	-3.72	2.15	-8.59	-8.99	4.44
(5.6)	-5.50	-5.71	3.67	-15.00	-15.40	2.60
(6.7)	-9.31	-9.79	4.90	-29.40	-30.86	4.73

[a] see Table 1

e: relative difference between calculated and experimental results

Table 4: Results of the simplex process

Temperature (°C)	Volume fraction of methanol θ	Separation factor α
48.00	0.80	1.00
45.00	0.79	1.00
47.10	0.75	1.01
45.10	0.73	1.02
45.20	0.70	1.03
47.40	0.65	1.04
48.00	0.60	1.06
50.00	0.57	1.07
45.94	0.57	1.13
47.96	0.59	1.10
43.00	0.60	1.14
40.10	0.61	1.24
35.00	0.57	1.30
31.41	0.52	1.32
30.95	0.50	1.33
27.00	0.50	1.34
26.00	0.52	1.37
25.00	0.52	1.37
27.00	0.51	1.35
25.00	0.50	1.36

An increase in the separation factor α with an increase in mobile phase polarity for all compound pairs explains that the separation conditions were obtained with low values of the methanol volume fraction in the methanol water mixture. To obtained, the most efficient separation conditions, a simplex method was used. This optimization process has been used in RPLC. Berridge [9] used the simplex algorithm for the optimization of mobile phase parameters in the reversed phase and ion pairing chromatography. Wang et al. [11] employed a mixture design simplex method for the computer-assisted optimization of the mobile phase, pH and/or ion concentrations. Guillaume [10, 12], used this process for the optimization of the mobile phase composition, column temperature and mobile phase flow rate in RPLC. Matsuda [13,

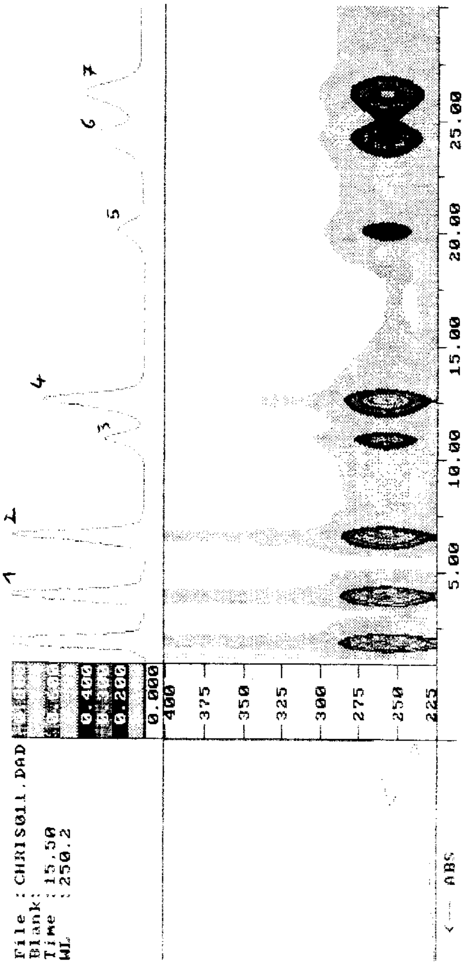

Figure 1: p-hydroxybenzoic esters chromatogram in the optimum conditions: methanol (52 % (v/v) in a methanol water mixture), column temperature: 25°C, flow rate: 0.90 mL/min. Number above peaks refers to the seven compounds. See Table 1.

14] demonstrated the advantages of a "function of mutual information (FUMI)" when used with the simplex algorithm for the optimization of mobile phase composition in RPLC.

For the simplex method developed in this experiment, the highest separation factor α was used as the separation criterion. In this procedure, α was calculated for three different starting conditions of the column temperature and mobile phase composition using equation 11.

The point corresponding to the lowest value of α is reflected in relation to the curve defined by the two other points to give a fourth set of starting conditions. Once again, the point with the lowest α is reflected and the process repeated until the same mobile phase composition and column temperature continue to be selected. The maximum α (1.37), the highest separation factor for the worst separated pair of peaks (IbuR, BuR) was determined after 19 operations made by the computer (Table 4). It can be noted that for $\theta \leq 0.50$, even if α increases and θ decreases, the computer stops running because the column efficiency decreases rapidly, the peak widths were too high and the analysis time was too long. The optimum conditions were obtained with an 0.52 volume fraction of water and a column temperature of 25°C. The chromatogram is given in Figure 1.

CONCLUSION

The retention of seven esters in HPLC was studied. Thermodynamic data were determined in a wide range of volume fractions of methanol θ in the methanol/water mixture. Experimental values were in agreement with those calculated by suitable retention models. These mathematical models associated with a simplex method separated the p-hydroxybenzoic esters and did not require any further experiments.

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