Journal of Chromatography A, 1217 (2010) 6052–6060

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

# Combined effects of mobile phase composition and temperature on the retention of homologous and polar test compounds on polydentate $C_8$ column

Pavel Jandera[a,*], Katarzyna Krupczyńska[b], Kateřina Vyňuchalová[a], Bogusław Buszewski[b]

[a] *Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, Studentská 573, CZ-53210 Pardubice, Czech Republic*
[b] *Chair of Environmental Chemistry and Bioanalytics, Faculty of Chemistry, Nicolaus Copernicus University, 7 Gagarin Str., PL-87-100 Toruń, Poland*

ARTICLE INFO

*Article history:*
Received 21 April 2010
Received in revised form 1 July 2010
Accepted 11 July 2010
Available online 16 July 2010

*Keywords:*
Temperature effects
Mobile phase
Retention model
Alkylbenzenes
Polydentate column

ABSTRACT

Combined effects of temperature and mobile phase on the reversed phase chromatographic behavior of alkylbenzenes and simple substituted benzenes were investigated on a Blaze $C_8$ polydentate silica-based column, showing improved resistance against hydrolytic breakdown at temperatures higher than 60 °C, in comparison to silica-based stationary phases with single attachment sites. For better insight into the retention mechanism on polydentate columns, we determined the enthalpy and entropy of the transfer of the test compounds from the mobile to the stationary phase. The enthalpic contribution dominated the retention at 80% or lower concentrations of methanol in the mobile phase. Entropic effects are more significant in 90% methanol and in acetonitrile–water mobile phases. Anomalies in the effects of mobile phase on the enthalpy of retention of benzene, methylbenzene and polar benzene derivatives were observed, in comparison to regular change in enthalpy and entropy of adsorption with changing concentration of organic solvent and the alkyl length for higher alkylbenzenes. The temperature and the mobile phase effects on the retention are practically independent of each other and – to first approximation – can be described by a simple model equation, which can be used for optimization of separation conditions.

## 1. Introduction

Even though much less used than in GC, temperature is an important parameter affecting separation efficiency, retention and selectivity in HPLC [1,2]. Using high temperature in HPLC is attractive as it offers faster analysis due to the reduction in mobile phase viscosity and enhanced solute diffusion. Further, it may improve the peak shapes, it needs less organic solvent in the mobile phase to accomplish the analysis and – last but not least – it allows alternative approaches to optimization of separation selectivity, which usually slightly decreases, but may more or less significantly change at elevated temperatures [3]. The beneficial effects of high temperature are especially advantageous in HPLC of large molecules [4,5]. In reversed-phase chromatography, a 4–5 °C increase in temperature causes a decrease in retention corresponding approximately to a 1% increase in concentration of methanol or acetonitrile [6], hence the temperature control is usually less effective a tool for controlling the retention than adjusting the composition of the mobile phase.

In recent years simultaneous optimization of temperature and mobile phase composition, or of the temperature and gradient profile in HPLC has been found useful in method development [7–11] and high temperature operation is attracting increased popularity in LC separations. The regulation of temperature is very convenient and simple, as it requires only a column thermostat allowing fast enough equilibration over the working temperature range. The operation at elevated temperature is facilitated by efficient preheating of the mobile phase to the column temperature.

In addition to the restrictions imposed by low boiling temperatures of some solvents and by the thermal sample instability, the main reason for relatively rare use of temperature as the operation parameter in HPLC is probably limited resistance of many silica-based chemically bonded stationary phases against hydrolysis at the temperatures higher than 60 °C, especially in aqueous mobile phases at pH below 3 or above 8. Hence, high-temperature HPLC applications are still rare and – until recently – have been restricted to stationary phases based on non-silica supports such as graphitized carbon, zirconium oxide based phases or polystyrene/divinylbenzene copolymers [4,12], which unfortunately usually show lower separation efficiency (HETP) then the silica-based column materials. Recently introduced silica-based polydentate reversed-phase materials with multiple-site bonded group attachment to the silica gel support show high efficiency and can be used even at temperatures over 100 °C [11]. It is believed that the polydentate bonded ligands may shield the surface of the silica gel support and protect it against hydrolysis at increased temperature.

---

* Corresponding author. Tel.: +420 46 6037023; fax: +420 46 6037068.
*E-mail address:* pavel.jandera@upce.cz (P. Jandera).

0021-9673/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2010.07.019

The type of the support and the chemistry of chemically bonded moieties have significant influence on the chromatographic properties of HPLC columns. However, in spite of the strong role of mobile phase in HPLC, simultaneous effects of the mobile phase and temperature on the retention mechanism have been relatively rarely studied in systematic manner [7–10]. The objective of the present study is the investigation of such combined effects on the retention of simple test compounds differing in polarities on a polydentate column with extended temperature stability, to elucidate possible differences in the retention mechanism at elevated temperatures. In the frame of this study, we focused attention on thermodynamic data and on the characteristics of various descriptors characterizing non-polar and specific polar contributions to the retention of homologous alkylbenzenes and benzene derivatives with various polar substituents.

## 2. Theoretical

### 2.1. The retention in homologous series

In reversed-phase HPLC with binary aqueous–organic mobile phases, the effect of the volume fraction of the organic solvent in the mobile phase, $\varphi$, on the logarithm of the retention factor, $k = (t_R - t_0)/t_0$, can be described – to first approximation – by a simplified equation (Eq. (1)) often used in practice for method development and optimization [13,14]:

$$\ln k = a - m\varphi \tag{1}$$

$t_R$ is the solute retention time, $t_0$ is the column hold-up time, $a$ is the logarithm of the retention factor extrapolated to pure water as the mobile phase and $m$ is the parameter characterizing the effect of the solvent on the retention (the solvent strength parameter).

In homologous or oligomer series, the parameters $a$ and $m$ of Eq. (1) regularly increase with increasing number of repeat monomer units, $n$ [15–18].

$$a = a_0 + a_1 n \tag{2}$$

$$m = m_0 + m_1 n \tag{3}$$

Introducing Eqs. (2) and (3) characterizing the effect of the number of repeat methylene units in the homologous series on the parameters of Eq. (1), Eq. (4) was derived to describe the retention in a homologous or oligomer series in binary mobile phases with varying composition [18]:

$$\ln k = a_0 + a_1 n - (m_0 + m_1 n)\varphi = \ln \beta + n \ln \alpha \tag{4}$$

Here, $\ln \alpha = (a_1 - m_1\varphi)$ is a measure of the repeat group (e.g., methylene) selectivity, i.e., the contribution of a methylene group to the energy of retention in a homologous series (in terms of $\ln k$) and $\ln \beta = (a_0 - m_0\varphi)$ characterizes the contribution of the non-repeat part of the molecule (the end-group) to the retention (such as, e.g., the phenyl group in the alkylbenzene series). The experimental parameters $a_1$, $m_1$ of Eq. (4) for homologous alkylbenzenes, can be used as a measure of the contributions of water and of the organic solvent, respectively, on the reversed-phase lipophilic (methylene) selectivity, and the parameters $a_0$, $m_0$, to characterize the contributions of water and of the organic solvent in the mobile phase on the end-group (phenyl) contributions to the $\ln k$ in binary aqueous–organic mobile phases [18].

### 2.2. Temperature effects – the enthalpy and entropy of retention

The retention in reversed-phase liquid chromatography usually decreases at elevated temperatures, presumably by reducing the difference in cohesive energy and hydrogen-bonding interactions between the mobile and the stationary phases. Other polar interactions are believed to be less affected by temperature variation in reversed-phase liquid chromatography, which therefore has the largest effect on the retention of compounds that differ in size and hydrogen-bonding basicity. Consequently, significant temperature effects on separation selectivity can be expected when the relative retention of two solutes is sensitive to conformation changes of the stationary phase at varying temperature, especially for partly ionized solutes or solutes significantly differing in the sizes or shapes of molecules, leading to differences in the entropy of retention [9].

The distribution constant $K = e^{-(\Delta G/RT)}$ depends on the change of the Gibbs energy connected with the solute retention, $\Delta G^0$, and is related to the corresponding changes of entropy, $\Delta S^0$, and enthalpy, $\Delta H^0$, of the transfer of the solute from the mobile to the stationary phase:

$$\Delta G^0 = -RT \ln K = \Delta H^0 - T\Delta S^0 \tag{5}$$

If a single retention mechanism controls the retention over a broad temperature range, the effect of temperature, on the retention factor, $k$, can be described by van't Hoff equation [19]:

$$\ln k = \ln K + \ln \frac{V_S}{V_M} = -\frac{\Delta G^0}{RT} + \ln \frac{V_S}{V_M} = \frac{\Delta S^0}{R} + \ln \frac{V_S}{V_M} - \frac{\Delta H^0}{RT} = A_i + \left(\frac{B_i}{T}\right) \tag{6}$$

In such a case, the $\ln k$ *versus* $1/T$ plots are linear, the slope parameter $B_i$ being proportional to the standard partial molar enthalpy of transfer of the solute $i$ from the mobile phase to the stationary phase, $-\Delta H^0$; the parameter $A_i$ includes the standard partial molar entropy of the transfer of the solute $i$ from the mobile phase to the stationary phase, $\Delta S^0$, and the phase ratio (the ratio of the volumes of the stationary, $V_S$, and of the mobile, $V_M$, phases) in the chromatographic system. $R$ is the gas constant and $T$ is the thermodynamic temperature (in Kelvin) [19–22]. Hence, by plotting $\ln k$ *versus* $1/T$ over a sufficiently broad temperature range, one may calculate the enthalpic and the entropic contributions to chromatographic retention and selectivity, $-\Delta H^0$ from the slope and $\Delta S^0$ from the intercept of the plot. Linearity or non-linearity of van't Hoff plots may provide information on whether or not the retention mechanism(s) may change over the studied temperature range [19,22–27]. As $\ln k$ increase in linear manner with $-\Delta H^0$ (Eq. (7)), the slope of the plot of $\ln k$ *versus* $\Delta H^0$, measured at several different temperatures, should be the same for various compounds, if a single mechanism controls the retention, such as in a homologous series [27].

The calculation of the entropic contribution to the retention from the intercept term, $A_i$, of Eq. (6) requires the numerical value of the phase ratio in the column to be known, which is not straightforward. The first problem is that it is generally impossible to determine correctly the boundary between the region occupied by the stationary and the mobile phase in the column, without adopting some convention [28]. The volume of the mobile phase in the column, $V_M$, may not correspond exactly to the column hold-up volume necessary for the calculation of the retention factor, $k$, which is usually determined from the elution time of a non-retained marker compound, $t_0$. The determination of the volume of the stationary phase, $V_S$, is even more complicated and controversial issue. Rigorous considerations relate $V_S$ to the surface area of the stationary phase, to the carbon content, the density or the mass of the stationary phase in the column. Neither quantity is directly measurable with a packed column, and calculations based on the data published by manufacturers (not always available) may not be reliable; further, batch measurements are time consuming and rarely possible, as few HPLC column manufacturers supply bulk materials. Hence, it seems that – even though theoretically not rigorous – the most