A general strategy for performing temperature-programming in high performance liquid chromatography—Prediction of segmented temperature gradients

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ABSTRACT

In the present work it is shown that the linear elution strength (LES) model which was adapted from temperature-programming gas chromatography (GC) can also be employed to predict retention times for segmented-temperature gradients based on temperature-gradient input data in liquid chromatography (LC) with high accuracy. The LES model assumes that retention times for isothermal separations can be predicted based on two temperature gradients and is employed to calculate the retention factor of an analyte when changing the start temperature of the temperature gradient. In this study it was investigated whether this approach can also be employed in LC. It was shown that this approximation cannot be transferred to temperature-programmed LC where a temperature range from 60 °C up to 180 °C is investigated. Major relative errors up to 169.6% were observed for isothermal retention factor predictions. In order to predict retention times for temperature gradients with different start temperatures in LC, another relationship is required to describe the influence of temperature on retention. Therefore, retention times for isothermal separations based on isothermal input runs were predicted using a plot of the natural logarithm of the retention factor vs. the inverse temperature and a plot of the natural logarithm of the retention factor vs. temperature. It could be shown that a plot of ln k vs. T yields more reliable isothermal/isocratic retention time predictions than a plot of ln k vs. $1/T$ which is usually employed. Hence, in order to predict retention times for temperature-gradients with different start temperatures in LC, two temperature gradient and two isothermal measurements have been employed. In this case, retention times can be predicted with a maximal relative error of 5.5% (average relative error: 2.9%). In comparison, if the start temperature of the simulated temperature gradient is equal to the start temperature of the input data, only two temperature-gradient measurements are required. Under these conditions, retention times can be predicted with a maximal relative error of 4.3% (average relative error: 2.2%). As an example, the systematic method development for an isothermal as well as a temperature gradient separation of selected sulfonamides by means of the adapted LES model is demonstrated using a pure water mobile phase. Both methods are compared and it is shown that the temperature-gradient separation provides some advantages over the isothermal separation in terms of limits of detection and analysis time.

1. Introduction

In recent years some high-temperature liquid chromatography (HT-HPLC) based hyphenation techniques [1–13] have been developed. These techniques require the use of a pure water mobile phase or the content of organic solvent in the mobile phase being as low as possible. In contrast to other approaches where temperature is usually discussed in terms of speeding up a separation [14,15] or increasing the efficiency [16,17], these hyphenation techniques make use of the fact that increasing the temperature of water results in a decrease in its static permittivity [18]. In other words, the higher the temperature of water, the lower the polarity of a pure water mobile phase. Therefore, whenever a mixture has to be separated where the polarity of the compounds is not too broad, temperature gradients can be employed instead of solvent gradients as has been shown elsewhere [7,19–21]. Hence, the practitioner is faced with the problem how to develop a method where temperature gradients are employed instead of solvent gradients. Most attempts to achieve adequate separations in temperature-programming mode under isocratic or solvent gradient conditions

are governed by trial and error [22–25], which also owes to the lack of sufficient prediction models. To overcome this problem, Nikitas and Pappa-Louisi developed retention models which permit prediction of retention times when solvent composition and temperature are changed simultaneously [26–28]. To date, their models were tested using only linear temperature gradients with moderate slopes from $2\,^\circ C\,min^{-1}$ up to $10\,^\circ C\,min^{-1}$ in a small temperature range from $15\,^\circ C$ up to $75\,^\circ C$ ($\Delta T = 60\,^\circ C$).

In a recent study [29] we could show that the linear elution strength (LES) model from temperature-programmed gas chromatography (GC) can be employed for retention time predictions for linear temperature gradients in temperature-programmed liquid chromatography (LC) under isocratic conditions. It was shown that retention times for temperature gradients with slopes up to $30\,^\circ C\,min^{-1}$ in a temperature range from $50\,^\circ C$ up to $180\,^\circ C$ can be predicted with high accuracy when the start temperature of the gradient is not changed. On the basis of these findings the aim of this work was to extend this model in order to predict retention times for more complex segmented temperature gradients in LC under isocratic conditions and to evaluate the errors in predicted retention times when the start temperature of the gradient is changed.

In high-temperature LC, the practitioner has the choice between an isothermal or a temperature-gradient operation mode. In order to reduce the experimental work during method development, it will be advantageous when only one basic data set is used for isothermal as well as temperature-gradient method development. In this context, the generation of temperature-gradient input data is less time consuming and requires less experimental work when compared to isothermal data acquisition. Moreover, in temperature-programming mode, samples containing analytes with different polarity can be measured within the same chromatographic run. In isothermal operation mode, the less polar compounds of the sample mixture will be retarded strongly at low temperatures, giving rise to very long retention times. Therefore, it would be advantageous if it is possible to predict isothermal as well as temperature-gradient separations based only on temperature-gradient input data, despite the fact that isothermal input data might be more precise and less affected by linear retention assumptions than temperature gradient input data [30]. Moreover, systematic method development of an isothermal as well as temperature-gradient separation of selected sulfonamides will be performed based on as few input measurements as possible. Finally, both operation modes will be compared.

2. Experimental

2.1. Chemicals

A test mixture of five sulfonamides consisting of sulfadiazine, sulfathiazole, sulfamerazine, sulfamethazine, sulfamethoxazole, and uracil was used. The analytes were dissolved in 75/25 (v/v) water/acetonitrile at a concentration of $50\,\mu g\,mL^{-1}$. Formic acid was employed to adjust the pH of the mobile phase to 2.7. All chemicals given above were purchased from Sigma–Aldrich (Seelze, Germany) in p.a. quality and used without further purification. High-purity water was prepared by an Elix 10-Milli-Q Plus water purification system (Millipore, Eschborn, Germany). Acetonitrile (ACN) was purchased from LGC Standards (Wesel, Germany) in Optigrade quality.

2.2. HPLC-system

A Shimadzu HPLC system (Shimadzu, Duisburg, Germany) was used which consists of two LC-10AD$_{VP}$ pumps, a DGU-14A degasser,
an SIL-10AD$_{VP}$ autosampler, an SPD-M10A$_{VP}$ diode array detector (DAD) and an SCL-10A$_{VP}$ controller. A 500 psi back pressure regulator (GammaAnalysenTechnik, Bremerhaven, Germany) was connected behind the DAD to keep the mobile phase in the liquid state. For data acquisition and analysis, Shimadzu LC solution (version 1.21 SP 1) was used.

2.3. Heating system

To heat the mobile and stationary phase a commercially available SIM HT-HPLC 200 high-temperature column oven (SIM – Scientific Instruments Manufacturer, Oberhausen, Germany) was used [31,32]. The heating system was specially designed for high-temperature liquid chromatography and consists of three modules, the eluent preheating unit, the column heating unit and the eluent cooling unit. The heat transfer is achieved by block heating which means that the capillaries and column are tightly enclosed by aluminium blocks. The three heating units can be controlled independently, which guarantees that the temperature of the mobile phase entering the column and the temperature of the stationary phase can be exactly matched. In order to compensate effects which are related to frictional heating, the practitioner can define a temperature difference between the eluent preheating and the column heating unit. If a temperature gradient is applied, the temperature of the preheating unit and the temperature of the column are increased simultaneously. For all measurements performed in this study, the temperature settings of the preheating unit and the column were identical.

2.4. Isothermal/isocratic measurements

The measurements were carried out on a Waters XBridge C18 (75×4.6 mm; $2.5\,\mu m$) column using a water mobile phase with 0.1% formic acid at a flow rate of $1.0\,mL\,min^{-1}$. For isothermal runs at $60\,^\circ C$ and $80\,^\circ C$ the injection volume was set to $2\,\mu L$ whereas for measurements from $100\,^\circ C$ up to $180\,^\circ C$ the injection volume was set to $1\,\mu L$. UV detection was carried out at a wavelength of $270\,nm$. The sampling rate and the time constant of the UV detector were set to $3.125\,Hz$ and $0.32\,s$, respectively.

2.5. Temperature-gradient measurements

Two temperature ranges from $60\,^\circ C$ up to $180\,^\circ C$ and $100\,^\circ C$ up to $180\,^\circ C$ were investigated. Temperature gradients with slopes from $2\,^\circ C\,min^{-1}$ up to $12\,^\circ C\,min^{-1}$ were applied. All other chromatographic conditions equal the respective isothermal measurements.

3. Theory

In a previous study [29] we have shown that the LES model could successfully be adapted from temperature-programmed gas chromatography to temperature-programmed liquid chromatography. Moreover, it was shown that it is not necessary to extend the LES model to consider a temperature-dependent delay time when a high-temperature column oven based on block heating is employed. Using the LES model the retention time t_R of an analyte can be predicted as a function of experimental conditions using Eqs. (1) and (2) [33,34].

$$t_R = \frac{t_0}{2.3b_T} \ln[e^{2.3b_T}(k_0 + 1) - k_0] \tag{1}$$

with

$$b_T = \frac{t_0 S_T \Delta T}{tG} \tag{2}$$