



Possibilities of retention prediction in fast gradient liquid chromatography. Part 3: Short silica monolithic columns



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ARTICLE INFO

Article history:

Received 17 April 2015

Received in revised form 16 July 2015

Accepted 16 July 2015

Available online 20 July 2015

Keywords:

Gradient elution

Modeling of chromatography

Monolithic columns

Fast separations

Two-dimensional chromatography

ABSTRACT

We studied possibilities of prediction of the gradient elution data for alkylbenzenes, flavones and phenolic acids on two short octadecyl silica gel monolithic columns, namely a Chromolith Flash C18, 25×4.6 mm, and a “new generation” Chromolith High Resolution C18, 50×4.6 mm, in fast 1–2 min gradients. With fixed short gradient times and varying gradient ranges of acetonitrile concentration in water, high flow rates of the mobile phase (3–5 mL/min) could be used. The gradient elution data were predicted from four gradient models based on two-parameter and three-parameter isocratic retention equations. Various gradient retention models can be used for prediction of chromatograms and optimization of separation within a fixed gradient time. A two-parameter log–log model introduced in 1974 and a three-parameter model introduced in 1980 provided slightly more accurate prediction than the Linear Solvent Strength (LSS) semi-logarithmic two-parameter model, most frequently used in reversed-phase LC. A three-parameter model introduced in 1978 provided slightly improved accuracy of prediction of gradient data with respect to two-parameter models, in contrast to another, more recent three-parameter empirical model introduced in 2010 (which failed for gradients starting at a non-zero concentration of acetonitrile). Both a longer (5 cm) and more efficient Chromolith HR column and a shorter (2.5 cm) slightly less efficient Chromolith Flash column provide useful separations in fast gradients (1–2 min) at high flow rates (3.5–5 mL/min), especially in second dimension of two-dimensional LC \times LC, in combination with HILIC separation on monolithic microcolumn in D1.

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1. Introduction

Short analysis times become important in modern HPLC practice with the impact on the productivity of analytical laboratories. Fast generic gradient methods are important for drug discovery screening, raw material analysis, impurity profiling, pharmacokinetic studies and final product stability tests. Under gradient conditions, the numbers of peaks that can be separated within a fixed time significantly increase in comparison with isocratic elution. Gradient elution is a “must” to encompass potentially large differences between the sample components in 1–2 min, or even shorter separation times, in the second dimension of on-line two-dimensional LC \times LC separations, where fast cycle frequency is imposed by a short time available for the separation of fractions transferred onto the second-dimension column [1].

Fast HPLC analysis can be accomplished on short very efficient UHPLC columns packed with sub-2 μ m particles at a cost

of very high operation pressures. Recently, we investigated the accuracy of prediction of gradient data in fast gradient chromatography (1–2 min) on short columns packed with fully porous [2] or superficially porous (core–shell) particles [3,4] with different chemistries of bonded stationary phases. We found that either 5 or 3 cm core–shell columns may provide comparable peak capacity in a fixed short gradient time with optimized gradient range.

In the present work, we extended the earlier investigation to two first and second generation silica-based monolithic C₁₈ columns as another approach to fast separation at moderate pressures. Monolithic columns consist of a single-piece continuous separation media (rods) [5]. The structure of monolithic media can be represented as a network of small mesopores, which are responsible for the retention and separation selectivity, interconnected by large flow-through pores. This dual pore-size morphology provides good bed permeability and low flow resistance [6]. The first-generation silica-based monolithic columns allowed approximately three times faster analyses at the same operating pressure and comparable separation efficiency to the columns of the same length packed with 5 μ m fully porous particles. Recently, new “second generation” Chromolith® HR columns with a tighter radial

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pore distribution were introduced with claimed 50% higher efficiency and longer lifetime compared with the standard Chromolith columns [7,8]. Gritti et al. [9] investigated the gradient elution performance of 3.2×50 mm second generation silica monolithic columns, which showed peak capacities similar to the core–shell Kinetex columns.

Recently, we have applied the theory of gradient elution for prediction of retention data and optimization of fast second-dimension gradients in two-dimensional LC \times LC [2,10–12]. Because of rapid changes in mobile phase composition during the fast gradients, short gradient times used in the repeated fraction transfer cycles impose that the column must be rapidly re-equilibrated to the initial conditions between the repetitive gradient analyses [13]. The re-equilibration can occur quickly, with less than three column volumes of conditioning solvent for flushing out the system dwell volume, V_D , and the column hold-up volume, V_M [14]. Recent investigation confirmed that gradient retention times are less sensitive to minor fluctuations in flow rate, temperature and mobile phase composition in comparison with isocratic data [15].

Gradient elution methods can be efficiently developed from an appropriate formal model, which however need not have any direct connection with the real retention mechanism, which may be rather complex. In the model equations, sample retention parameters are needed, which can be acquired in independent initial gradient or isocratic experiments. The most frequently used commercial optimization software, DryLab, employs two initial scouting gradients, a short one and a longer one, to find the optimum time of a simple (or segmented) gradient, yielding best resolution of a particular sample [16]. Running a few isocratic experiments with varying concentration of the strong solvent in the mobile phase is also suitable for the acquisition of the necessary input parameters to predict gradient behavior in a wide range of combinations of the adjustable experimental gradient parameters (flow rate, starting and final gradient concentrations, the volume of the mobile phase in the gradient, etc.). It is most important that the mobile phase composition range used for either isocratic or gradient parameter determination covers sufficiently broad range of not too low retention, with $k > 0.5$.

The equilibrium distribution between the stationary and the mobile phases is directly proportional to the retention factor, k , which is constant in isocratic chromatography, but continuously changes during gradient elution. If the instrumental gradient profile passes unchanged through the column (the gradient profile is not distorted) during the gradient run, the errors of predicted gradient data can be attributed to the errors in determination of the model parameters, irrespective of the elution mode (gradient or isocratic) used for their acquisition. However, there may be several non-thermodynamic sources of the deviations of the actual gradient profile, namely due to preferential adsorption on the column, rounding of the starting and of the final gradient parts, instrumental gradient delay function of the gradient mixer, etc., which may be affected by the model parameters when the gradient mode is used for their acquisition, unlike to isocratic parameter acquisition. Fast steep gradients on short columns studied in this work are probably more liable to these errors than the longer ones.

The best way to find the answer is to compare the experimental retention data with the data predicted from a model, which is not affected by the particular kinetic effects of fast gradients. For this purpose, models with parameters acquired during gradient elution would not be as useful as the calculations with isocratic parameters, as the thermodynamics of retention is the same under both isocratic and gradient conditions. Hence deviations caused by non-thermodynamic sources in gradient elution should be clearly apparent from the discrepancies between the predicted and experimental gradient retention data.

In reversed-phase chromatography (RPLC), a simple semi-logarithmic (Linear Solvent Strength, LSS) isocratic model has been widely used to describe the effect of the volume fraction of the organic solvent, φ , on the retention factor, k , in aqueous-organic mobile phases [17]:

$$\log k = a - m \cdot \varphi \quad (1)$$

The parameters a (extrapolated $\log k$ in water) and m (the organic solvent strength parameter, sometimes denoted as S [16,18]) depend on the solute, stationary phase and type of the organic solvent.

Another simple log–log two-parameter Eq. (2) was originally introduced to describe the effects of the concentration of a more polar solvent in a less polar one, φ , on the retention factors, k in normal-phase separation systems with mixed organic mobile phases [17,19]:

$$\log k = a - m \cdot \log \varphi \quad (2)$$

k_0 , a and m are experimental constants, k_0 being the retention factor in the pure strong solvent.

Various three-parameter model equations were employed to describe the effects of the mobile phase on retention [17,21–25]. These models have been reviewed and compared earlier elsewhere [25–28]. The most frequently used has been the second-order polynomial isocratic model published by Schoenmakers [20] (in fact, we published essentially the same model earlier, see [17]).

One of the first three-parameter models introduced a third parameter, b , into Eq. (2) to account for possible weaker retention of some samples in the less strong solvent [21]:

$$k = (b + a \cdot \varphi)^{-m} \quad (3)$$

Here, $1/(b)^m$ is the k in water ($\varphi = 0$). For very low b , Eq. (3) becomes identical with Eq. (2).

Recently, Neue and Kuss introduced an empirical model using three-parameters (k_{00} , b , a) [29]:

$$k = k_{00}(1 + a \cdot \varphi)^2 \cdot e^{-b \cdot \varphi / (1 + a \cdot \varphi)} \quad (4)$$

k_{00} is k in pure water and a accounts for weaker retention in water. For very low a Eq. (4) becomes identical with Eq. (1). In this article, some parameters of Eqs. (4), (9) and (15) were changed to avoid possible confusion with the terms traditionally used in the earlier literature: B was changed to b , c to φ , c_0 to A , S to B , t_0 to t_m .

In the present work, we employ four isocratic models described by Eqs. (1)–(4) as the source of the model parameters for the calculations of gradient elution data for alkylbenzenes, phenolic acids and flavones in fast (1 min) gradients of acetonitrile in water starting at 0% and higher concentrations of acetonitrile, on short silica monolithic columns. Further, we investigate the effects of the gradient range on the peak capacity and we report new methods of the determination of the column efficiency in fast gradient elution. To our best knowledge, there has been no such systematic study of fast gradients on monolithic columns performed so far.

2. Theory

2.1. Prediction of gradient elution times (volumes) from the isocratic retention data

In linear gradient reversed-phase chromatography, the volume fraction of a polar organic solvent (acetonitrile, methanol) in water, φ , increases with the volume of the mobile phase that has flowed through the column in time t from the start of the gradient, $V = t F_m$:

$$\varphi = A + B \cdot V \quad (5)$$

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