



Very high pressure liquid chromatography using core-shell particles: Quantitative analysis of fast gradient separations without post-run times



Joseph J. Stankovich, Fabrice Gritti, Paul G. Stevenson, Lois A. Beaver, Georges Guiochon*

Department of Chemistry, University of Tennessee, Knoxville, TN, USA

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ABSTRACT

Five methods for controlling the mobile phase flow rate for gradient elution analyses using very high pressure liquid chromatography (VHPLC) were tested to determine thermal stability of the column during rapid gradient separations. To obtain rapid separations, instruments are operated at high flow rates and high inlet pressure leading to uneven thermal effects across columns and additional time needed to restore thermal equilibrium between successive analyses. The purpose of this study is to investigate means to minimize thermal instability and obtain reliable results by measuring the reproducibility of the results of six replicate gradient separations of a nine component RPLC standard mixture under various experimental conditions with no post-run times. Gradient separations under different conditions were performed: constant flow rates, two sets of constant pressure operation, programmed flow constant pressure operation, and conditions which theoretically should yield a constant net heat loss at the column's wall. The results show that using constant flow rates, programmed flow constant pressures, and constant heat loss at the column's wall all provide reproducible separations. However, performing separations using a high constant pressure with programmed flow reduces the analysis time by 16% compared to constant flow rate methods. For the constant flow rate, programmed flow constant pressure, and constant wall heat experiments no equilibration time (post-run time) was required to obtain highly reproducible data

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1. Introduction

Over time and particularly over the last ten years, manufacturers of chromatographic instruments and columns have considerably improved technology. Shorter columns are packed with finer particles and are operated at higher velocities than they were ten years ago, providing higher column efficiencies, which reduce the analysis times. These trends persisted for fifty years but are now reaching a limit. The permeability of columns packed with fine particles decreases rapidly with decreasing particle size; these columns need to be operated with high inlet pressures to achieve high column efficiency. This leads to a new source of difficulties due to mobile phase expansion and its friction against the column bed. These effects generate heat that diffuses along and across the column, affecting the temperature distribution through the column bed. While still negligible for 20 cm long columns packed with 5 μm particles and eluted under an inlet pressure of a few hundred bars, this heat is

considerable for 5 cm long columns packed with 1.7 μm particles and eluted under an inlet pressure of 1000 bar [1–15]. The column is no longer isothermal, the mobile phase viscosity, the analyte diffusion coefficients, and the analyte retention factors are no longer constant along and across the column. For analyses carried out under isocratic conditions, the heat exchanges between the bed and the column environment may be limited by keeping the column in a still air enclosure. Then, the temperature gradient is radially isothermal, which minimizes the radial gradients of mobile phase viscosity and velocity, and of analyte retention factors. However, analysts experience the consequences of axial gradients of these properties.

During gradient analyses, the mobile phase composition varies; its viscosity and the relevant analyte properties change as well. Accordingly, the heat power generated by the percolation of the mobile phase stream varies during the analysis and its distribution across the column changes depending on the delay between successive analyses. Reproducible results can be achieved when the frequency of analyses is sufficiently low to allow the column to return to thermal equilibrium between successive analyses. The analysis time and the delay between injections contribute to the throughput [10–12], so increasing the equilibration time is inconsistent with

* Corresponding author at: University of Tennessee, Department of Chemistry, Knoxville, TN 37996-1600, USA. Tel.: +1 8659740733; fax: +1 8659742667.

E-mail address: guiochon@utk.edu (G. Guiochon).

Table 1
Summary of experimental conditions and peak information.

Solvent gradient 50–95% acetonitrile in water for all experiments					
Experiment set	Constant flow	Constant pressure 1	Constant pressure max.	Programmed flow constant pressure max.	Constant wall heat
Gradient time	2.1 min	2.15 min	1.85 min	1.85 min	1.7 min
Total time	2.25 min	2.25 min	1.9 min	1.9 min	2.25 min
Flow rate	2.8 mL/min	Variable	Variable	Variable	Variable
Pressure	Variable	435 bar	510 bar	≈510 bar	Variable
Retention time RSD	0.029%	0.32%	0.35%	0.057%	0.029%
Peak width RSD	2.9%	0.69%	0.88%	0.89%	2.0%
Retention time RSD (first separation excluded)	0.018%	0.099%	0.060%	0.026%	0.027%
Peak width (first separation excluded)	0.97%	0.66%	0.69%	0.55%	1.79%
Peak capacity	31.4	25.9	28.0	29.2	23.2
Peak capacity per min of total run time	14.0 (peaks/min)	11.5 (peaks/min)	14.7 (peaks/min)	15.4 (peaks/min)	10.4 (peaks/min)

the purpose of achieving fast analyses. Decreasing analysis times by increasing the flow rate beyond the optimal flow rate of the column decreases the net peak capacity and increases the frictional heat power of the mobile phase stream. Better solutions are needed.

While gradient separations are traditionally carried out at a constant flow rate, several alternate approaches were recently proposed to accelerate these separations in VHPLC. Constant pressure gradient [13] and constant temperature at the column wall [14] were suggested. Based on the theoretical and potential advantages of gradient separations performed under steady state temperature, our goal is to explore and compare several approaches to optimize separation throughputs in VHPLC and to determine the reproducibility of the results.

2. Material and methods

2.1. Instruments, columns, and reagents

Experiments were conducted in a 24°C room using a prototype Agilent 1290 infinity system capable of constant pressure operations and equipped with a low volume (600 nL) detector flow cell (Agilent Technologies, Waldbronn, Germany). Two thermo viper (130 μm I.D., 250 mm long) column connection units (Thermo Fisher, Waltham, MA, USA) were used, one to connect the column to the injector, and the other to connect the column to the detector. A 4.6 × 100 mm Agilent technology column packed with Poroshell 120 EC-C18 particles (2.7 μm size) was used for all experiments.

Fisher scientific (Fair Lawn, NJ, USA) HPLC grade water and acetonitrile were used. The test mixture was composed of (in order of elution) acetanilide, acetophenone, propiophenone, butyrophenone, benzophenone, valerophenone, hexanophenone, heptanophenone, and octanophenone, all at 100 μg/mL (±0.5%) (RPLC checkout sample Agilent Technologies P/N 5188-6529) in a solution of acetonitrile/water (65:35 v/v). 1 μL was injected for the performance evaluation.

2.2. Experimental conditions

All the experiments were performed using the briefest equilibration periods (post-run times) that the instrument would allow. Six consecutive 1 μL injections of the standard solution were performed for each set of experiments. For the constant flow, constant pressure, and programmed flow constant pressure experiments, the column was positioned horizontally and insulated in several layers of cellulose sheets to buffer the changes in room temperature due to the heating/cooling system in the room. For the constant heat loss experiments, the column was positioned in the oven, with the door removed. Exposure to open air is necessary for constant wall heat experiments; the equations used to derive the appropriate conditions taking into account the diffusion of heat into open

air. The column was operated for 15 min before the first separation was made in each series.

Constant flow experiments were input into the ChemStation software in the conventional way. Constant pressure operation is controlled by the instrument pumping system, based on the inlet pressure of the column throughout the gradient. The time of the gradient is based on the net volume eluted, lower pressures would elute the mobile phase at a slower rate and therefore the net time required would be greater than under a higher pressure. Programmed constant pressure operation is based on the values of the flow rate and the gradient composition each 3 s increments derived from the constant pressure operation (at 510 bar). In programmed constant pressure operation the instrument controls the flow rate and gradient compositions based on inputs set by the operator in 3 s increments, regardless of the actual inlet pressure of the column. The constant heat loss gradient is operated in a similar manner where the results of several equations are used to generate a gradient which should keep the net heat dissipated by the column's wall constant, as is discussed in greater detail in a following section. Time based chromatograms are recorded and shown in this work.

2.2.1. Constant flow rate experiments

Pressure varied while a constant gradient flow rate of 2.8 mL/min was maintained for 2.1 min with a total runtime of 2.25 min (Table 1). The constant flow rate gradient was input into the ChemStation software in conventional operation.

2.2.2. Constant pressure experiments

Constant pressure experiments were conducted using the constant pressure interface of the Agilent 1290 that controls the mobile phase flow rate based on the inlet pressure set by the operator. For the constant pressure experiments the instrument uses a volume based gradient, so the net volume of solvent eluted for all separations are the same. This volume based gradient is based on the constant flow conditions, where the constant flow method is converted to a constant pressure method by use of an instrument macro. The macro instructs the instrument to change the solvent composition of the mobile phase as a function of net volume of solvent eluted for the constant pressure methods. For the constant pressure and programmed constant pressure experiments the variable is how the volumetric gradient is delivered, either by instrument controlled pressure, or manual input of the gradient steps in the instrument software.

Two series of constant pressure experiments were performed (see Table 1). The first one (constant pressure 1) had the inlet pressure set to 435 bar. This pressure was selected to give the same net analysis time as the constant flow experiments. After one constant

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