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Extending the limits of operating pressure of narrow-bore column liquid chromatography instrumentation

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ABSTRACT

The increase of the operating pressure in Liquid Chromatography, has been one of the crucial steps toward faster and more efficient separations. In the present contribution, it was investigated if the pressure limits for narrow-bore columns (2.1 mm ID) could be increased beyond those of commercially available (1300 bar) instrumentation without performance loss. Whereas previous studies applying pressures higher than 2000 bar were limited to the use of columns with a diameter smaller or equal to 1 mm, it is a difficult feat to expand this to 2.1 mm ID given that viscous-heating effects increase according to the fifth power of the column radius. A prototype LC set-up was realized, allowing to operate at pressures up to 2600 bar (260 MPa) for large separation volumes (>5 mL). The performance of an in-house-built injector was compared at 800 bar to commercially available injectors, yielding equal performance but twice the maximum pressure rating. The performance of (coupled) custom columns packed with fully porous and superficially porous particles were assessed at ultra-high-pressure conditions. Increasing the inlet pressure from 800 to 2400 bar and scaling the column length proportionally (from 150 mm to 450 mm), resulted in the theoretically expected linear increase in plate count from 20,000 to 59,000. A maximum plate number of 81,000 was realized using a 600 mm long (coupled) column at 2600 bar. Viscous-heating effects were diminished by insulating coupled columns and applying an intermediate-cooling strategy in a forced-air oven.

1. Introduction

To achieve faster and better separations, reducing the particle diameter provides a time-proven method. However, columns packed with small particle sizes can only be operated around the optimal velocity if the maximal operating pressure for liquid chromatography (LC) devices is sufficiently high. So-called ultra-high-pressure instrumentation is now widely available from different instrument manufacturers, but an upper pressure limit in operating pressure appears to have been reached around 1200–1300 bar due the mechanical challenges in the construction of reliable instrumentation and problems with column bed stability and lifetime. Additionally, concerns arose regarding the effect of viscous heating which leads to radial temperature and thus velocity profiles, that in turn result in additional band broadening. The latter effects have extensively been described nearly 3 decades ago [1–4] and received a renewed attention with the introduction of ultra-high-pressure liquid chromatography (UHPLC) [5–13].

In the most simplified approach, the efficiency of a chromatographic system can be described by the Van Deemter equation for the height equivalent of a theoretical plate (H)

$$H = A + \frac{B}{u_0} + C \cdot u_0 \tag{1}$$

where A, B and C are the coefficients for eddy diffusion, longitudinal diffusion and resistance to mass transfer and u_0 is the linear velocity. Since the A- and C-term are respectively proportional to the particle diameter (d_p) and to d_p^2, smaller particle diameters will lead to lower plate heights and thus better separations. In addition, the optimal linear velocity increases with the inverse of the particle diameter. However, the pressure drop across the column increases with the square of the inverse particle diameter according to

$$\Delta P = \frac{\Phi \cdot L \cdot u_0 \cdot \eta}{d_p^2} \tag{2}$$

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where ΔP, Φ, L and η are, respectively, the pressure drop along the column, the flow resistance, the column length and the viscosity of the mobile phase. Thus decreasing the particle diameter, results in an increase in pressure drop over the column proportional to the third power of the inverse particle diameter at the optimal flow rate, as $u_{0,\mathrm{opt}}$ is proportional with $1/d_p$. The power dissipated in the liquid when flowing through a packed bed, can be expressed by [15]

$$Power = \Delta P \cdot F \tag{3}$$

where F is the volumetric flow rate. As a result, reducing the particle size will lead toward an increase in heat generation with the fourth power of the particle size at optimal flow rate, F_{opt} (as $F_{\mathrm{opt}} \sim u_{0,\mathrm{opt}} \sim 1/d_p$).

Several possibilities have already been proposed for coping with the increased heat dissipation that occurs at ultra-high pressures (pressures beyond 2000 bar [15]) such as the use of intermediate cooling [13], superficially porous particles [14], or capillary columns [15–19]. In the case of intermediate cooling, the column is split up in n shorter columns and the heat is removed in the intermediate connection capillaries. As a result, the pressure drop over each column is limited to $\Delta P/n$, and hence the temperature increase in each column is almost n-times smaller [13]. Since columns packed with superficially porous particles have a higher thermal conductivity, the heat can be removed more efficient radially, thus alleviating to some extent the occurrence of temperature and velocity profiles. For capillary columns, the large surface to volume ratio allows efficient dissipation of the generated heat.

Originally, the term ‘ultra-high pressure’ was introduced by Jorgenson and co-workers [15–19] in their seminal work on capillary LC instrumentation capable of working at pressures in the range of 4000–6000 bar, a 10-fold increase versus state-of-the art commercial instruments at that time. However, with the introduction of ultra-performance liquid chromatography (UPLC) by Waters and the adaption of related terms such as “ultra-high performance” or “ultra-high pressure” liquid chromatography (UHPLC) for instrumentation with upper pressure limits between 800 and 1300 bar, the meaning of ultra-high has become obscured. It is important to stress that the term ultra-high pressure is used in the current contribution for a system with an operating pressure of 2600 bar, i.e. twice that of the best commercial system. To avoid confusion, the in-house built system will be referred to as UHPLC*.

In the work by Jorgenson and co-workers capillary columns were used to cope with the viscous heating effect at ultra-high pressures. This has extensively been examined up to 7000 bar for isocratic as well as gradient elution [15–18]. When lowering the inner diameter of the column from 4.6 mm to 50 μm, the heat generated will decrease with a factor of more than 2100 [19]. This lower heat generation in combination with the efficient heat dissipation to the column surroundings, result in negligible radial temperature profiles. Other research was performed at operating pressures above 2000 bar in capillary LC by Lee and coworkers and Colon and coworkers [20–22].

In addition, Colon and coworkers reported on the use of microbore columns (1 mm ID) at pressure up to 1400 bar [23–25]. These authors also investigated the use of 1.5 mm ID columns at higher operating pressures (up to 2000 bar), but the main focus of this study was on the repeatability of an injector system and did not discuss the performance or retention behavior in detail [23]. As the effects of viscous heating on performance, that are predominant when using ultra-high pressure in columns with a large ID, can increase with the fifth power of the column ID, the shift from 1 mm to 2.1 mm is not straightforward [26]. Using 2.1 mm ID columns is however highly relevant in LC since narrow/normal-bore columns [27] (2.1–4.6 mm) still account for the majority of the market (>75%) [28]. This study aims at illustrating the possibilities and limitations of ultra-high pressures for these column dimensions. The primary goal of the study is to identify if performance can be scaled with column length and thus inlet pressure, in order to assess the possible limiting effects of viscous heating. In addition, temperature profiles were monitored to investigate the application of intercooling at UHPLC*-conditions. However for investigating narrow-bore columns, several experimental challenges need to be resolved, including:

- The significant effect of viscous heating effects, as mentioned above.
- The separation volume ($V_{\mathrm{sep,HPLC}}$) needed, i.e. the total volume of solvent needed to elute all components in a separation, is much higher than the internal pump volume (V_{pump}) and is much higher than in capillary LC ($V_{\mathrm{sep,Cap\ LC}}$): $V_{\mathrm{sep,HPLC}} \gg V_{\mathrm{pump}} \gg V_{\mathrm{sep,Cap\ LC}}$. This means that when only one pump is used (as in previous work in UHPLC*-conditions [15–18,20–24]), the system experiences large pressure drops at the end of each pump cycle, when the internal volume of the pump is refilled. These abrupt pressure changes are detrimental for a stable operation (flow rate, detector signal) and can have adverse effects on the stability of the column packing.
- Mechanical deformation and stability of column hardware and packing material.
- Safety issues at ultra-high pressures.

2. Experimental system

2.1. Columns and chemicals

Prototype columns (packing and hardware), that were packed applying a maximal pressure of 3000 bar, were provided by Thermo Scientific: Accucore (superficially porous C18, 150 mm × 2.1 mm, 2.6 μm) and Hypersil columns (fully porous C18, 150 mm × 2.1 mm, 1.9 μm).

Uracil (t_0-marker) and a mixture of 4 parabens (methyl-, ethyl-, propyl- and butyl-paraben) were purchased from Sigma–Aldrich (Steinheim, Germany). All the test components were dissolved in a mixture of 50/50 v% Methanol/H_2O. Methanol (Supra-Gradient grade) was purchased from Biosolve (Valkenswaard, Netherlands). HPLC grade water was prepared in the laboratory using a Milli-Q gradient water purification system (Millipore, Bedford, MA, USA).

2.2. Liquid chromatograph set-up for ultra-high pressures

Fig. 1 gives a schematic overview of the set-up: two three-stage air-actuated pneumatic amplifier liquid pumps (model DSXHF-903, Haskel, Burbank, CA) were used to generate pressures above 2000 bar. The amplification ratio of the pumps is 903, meaning that applying a compressed air pressure of e.g. 4 bar results in a liquid pressure of 3612 bar. The maximal liquid pressure for the pumps is 5171 bar. The two liquid streams join together in a T-piece (model 60T4 1/4 in. H/P) and then passes through a cross-piece (model 60X4 1/4 in. H/P) where a rupture disk holder (model 60-SH4-1/4A Angled Disk) and pressure sensor (model TPHADA, Gefran, Italy) are installed. The rupture disk was custom-made to the desired burst pressure. All high-pressure tubing (gray in Fig. 1), connections and parts were purchased from Butech, Spain. The system air-drive pressure is controlled by a first pressure controller with filter (model Olympian Plus B64G-4GK-AD3-RMN, Norgren), after which the flow was splitted into a channel to drive the injector valves and a channel to control the air-drive pressure for both pumps. The latter channel is once again split into two separate channels with two separate air pressure controllers (model 61B2/BT000, 5–10 bar, Norgren) for both pumps, each equipped with a manometer to read out the air-drive pressure. The first air-drive pressure controller is