Journal of Chromatography A, 1523 (2017) 17–39

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Review article

Recent advances in capillary ultrahigh pressure liquid chromatography

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ARTICLE INFO

Article history:
Received 15 March 2017
Received in revised form 12 May 2017
Accepted 15 May 2017
Available online 17 May 2017

Keywords:
Capillary LC
Ultrahigh pressure liquid chromatography
nanoLC
Stationary phases
LC columns
Proteomics

ABSTRACT

In the twenty years since its initial demonstration, capillary ultrahigh pressure liquid chromatography (UHPLC) has proven to be one of most powerful separation techniques for the analysis of complex mixtures. This review focuses on the most recent advances made since 2010 towards increasing the performance of such separations. Improvements in capillary column preparation techniques that have led to columns with unprecedented performance are described. New stationary phases and phase supports that have been reported over the past decade are detailed, with a focus on their use in capillary formats. A discussion on the instrument developments that have been required to ensure that extra-column effects do not diminish the intrinsic efficiency of these columns during analysis is also included. Finally, the impact of these capillary UHPLC topics on the field of proteomics and ways in which capillary UHPLC may continue to be applied to the separation of complex samples are addressed.

Contents

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http://dx.doi.org/10.1016/j.chroma.2017.05.039
0021-9673/

1. Introduction

For approximately 40 years, liquid chromatography (LC) systems with a 400 bar pressure limit were the mainstay for liquid-phase separations. To meet the analytical demands of more complex samples, columns packed with smaller diameter particles were introduced, although the column length was also reduced to stay below this pressure limit [1]. This decrease in column length negated the theoretical efficiency gains that could be attained with smaller particles. To enable higher efficiency separations with smaller particles, ultrahigh pressure liquid chromatography (UHPLC) which utilized pressures up to 4000 bar was introduced in 1997. In these initial experiments, sub–2 μm non-porous silica particles were packed into 50–70 cm long capillary columns with observed minimum plate heights of 2.1 μm [2]. The use of capillary columns rather than the standard 4.6 mm i.d. (inner diameter) columns provided improved efficiency, especially at the smallest i.d. values, as well as minimized band broadening due to frictional heating at ultra-high pressures [1]. In the several years that followed, further research into UHPLC instrumentation and column design was reported [3–16]. During this early phase of UHPLC development, inlet pressures exceeding 7000 bar and optimal column efficiencies over 700,000 plates/m were achieved [4]. The key result of this early work was the release of the first commercial UHPLC instrument in 2004 [17], which increased the use of the technique in the separations community. With the introduction of commercially available UHPLC instrumentation, the technology was quickly adopted by a wide range of industries [18]. As UHPLC methodology has matured, the instrumentation has moved beyond research laboratories to routine testing facilities and use of ultrahigh pressures with 2.1 mm i.d. columns has become common. Although these columns tend to be more robust, moving to the capillary scale can lead to improvements in sensitivity and reductions in solvent consumption. With this further reduction in column diameter, specialized instrumentation with very low system volume is required to ensure that column efficiency is not reduced by extra-column band broadening.

An in-depth review on the theory and practice of capillary UHPLC was published in 2010 by Jorgenson [1]. In that paper, the motivations behind using smaller particles as LC stationary phase supports to obtain better chromatographic efficiency, and the higher pressures needed to use columns packed with such particles, were detailed. In the time since, a number of other review articles focused on general UHPLC [18–24] and capillary-scale LC [25–27] have followed, but none have completely highlighted the overlap of the two. Thus, this review details a number of significant advancements in capillary UHPLC since 2010 related to column preparation and performance, stationary phases and support materials, instrument technology, and the application of the technique to investigate areas of biological relevance.

2. Capillary UHPLC column preparation and theory

The driving motive behind the investigations which eventually led to the commercial introduction of UHPLC was the promise of better separations in less time [1]. Chromatographic theory predicts that the use of smaller particles reduces the eddy dispersion (A) and mass transfer (C) terms of the van Deemter equation, each of which is proportional to particle size [28]. When considering performance in terms of a set pressure limit, kinetic (or Poppe-style) plots can be used to compare different operating conditions to determine the maximum efficiency that can be achieved [29,30]. In Fig. 1, three curves are shown on a single plot indicating three operating regimes: HPLC (3 μm particles, 350 bar maximum pressure), commercial UHPLC (1.9 μm particles, 1000 bar maximum pressure), and typical conditions reported for long, capillary-scale columns (1.5 μm particles, 3100 bar maximum pressure). For a given column void time (t_m = 100 s), the achievable plate count (and associated column length) for each of the particle sizes at the given pressure limit is 30,000 plates (22.1 cm) for the 3 μm particles, 65,000 plates (24.3 cm) for the 1.9 μm particles, and 108,000 plates (33.2 cm) for the 1.5 μm particles. However, with the use of pressures in excess of the 400 bar maximum associated with traditional HPLC, it is important to consider the thermal consequences of pumping mobile phase through a packed bed of sub–2 μm particles. Heat, generated through viscous dissipation, is removed from a column by two means: it flows axially to exit along with the mobile phase at the column outlet and radially through the column walls [31–33]. Accordingly, temperature gradients are established both axially and radially within the column. Radial temperature gradients, characterized by warmer mobile phase at the center of the column, contribute to chromatographic band broadening in two ways – both of which work in the same direction. First, the warmer, less viscous fluid at the center of the column flows faster than the cooler liquid at the column walls. Second, the temperature-dependent analyte partition coefficient is smaller at the warmer column center than at the cooler walls.

Power, the rate at which heat is generated within a packed bed, is equal to the product of the column operating pressure and the mobile phase flow rate [1]. In larger bore columns with higher flow rates, heat is generated at rates greater than that which is effectively dissipated through the column walls. Capillary columns, with their associated lower flow rates, generate heat at rates which are much more capably dissipated without severe detriment to band spreading. For a 50 cm long UHPLC column packed with 1 μm particles and operated at 7000 bar, the rate of heat generation is over 8000 times lower for a 50 μm i.d. column than for a 4.6 mm i.d. column [1]. Thus, capillaries provide the best platform for liquid chromatography performed at ultrahigh pressures for reducing thermal broadening.

The performance of capillary UHPLC columns with diameters ranging from 10–150 μm packed with both nonporous and porous materials ranging between 1 and 2 μm has been studied extensively [1,4,5,34]. In Fig. 2, capillary i.d. is shown to influence column efficiency with performance improving as inner diameter decreases [4]. Analyses of each of the van Deemter terms as a function of column diameter revealed the A term to improve proportionally with decreasing column diameter. This trend was explained as the result of analyte molecules more effectively sampling all of the available flow paths within the column by transverse diffusion as diameter decreased. The C term was also observed to improve with decreasing column diameter. Considering that all the columns were packed using the same 1 μm nonporous particles, the reasons for this improvement were not readily apparent through analysis of the van Deemter model of chromatographic band broadening.

The van Deemter equation groups the contributions to band broadening into three independent terms according to their dependencies on mobile phase linear velocity (u_{avg}) [22]. The reduced