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Comparison of ultra-high performance supercritical fluid chromatography and ultra-high performance liquid chromatography for the analysis of pharmaceutical compounds

Alexandre Grand-Guillaume Perrenoud, Jean-Luc Veuthey, Davy Guillarme*

School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Boulevard d'Yvoy 20, 1211 Geneva 4, Switzerland

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ABSTRACT

Currently, columns packed with sub-2 μm particles are widely employed in liquid chromatography but are scarcely used in supercritical fluid chromatography. The goal of the present study was to compare the performance, possibilities and limitations of both ultra-high performance liquid chromatography (UHPLC) and ultra-high performance supercritical fluid chromatography (UHPSFC) using columns packed with sub-2 μm particles. For this purpose, a kinetic evaluation was first performed, and van Deemter curves and pressure plots were constructed and compared for columns packed with hybrid silica stationary phases composed of 1.7 and 3.5 μm particles. As expected, the kinetic performance of the UHPSFC method was significantly better than that of the UHPLC. Indeed, the $h_{\min}$ values were in the same range with both strategies and were between 2.2 and 2.8, but u_{opt} was increased by a factor of >4 in UHPSFC conditions. Another obvious advantage of UHPSFC over UHPLC is related to the generated backpressure, which is significantly lower in the presence of a supercritical or subcritical fluid. However, the upper pressure limit of the UHPSFC system was only ~400 bar vs. ~1000 bar in the UHPLC system, which prevents the use of highly organic mobile phases at high flow rates in UHPSFC. Second, the impact of reducing the particle size (from 3.5 to 1.7 μm) was evaluated in both UHPLC and UHPSFC conditions. The effect of frictional heating on the selectivity was demonstrated in UHPLC and that of fluid density or decompression cooling was highlighted in UHPSFC. However, in both cases, a change in selectivity was observed for only a limited number of compounds. Third, various types of column chemistries packed with 1.7 μm particles were evaluated in both UHPLC and UHPSFC conditions using a model mixture of acidic, neutral and basic compounds. It has been shown that more drastic changes in selectivity were obtained using UHPSFC columns compared to those obtained by changing UHPLC columns. In addition, there was a good complementarity between the two separation modes. Finally, by combining the use of small particles with supercritical fluids as a mobile phase, it was possible to achieve the analysis of pharmaceutical compounds in less than 1 min or to attain a peak capacity of more than 250 in approximately 40 min, both with a high degree of repeatability.

1. Introduction

During the last decade, there have been significant advances in the instrumentation and columns for liquid chromatography (LC), which consequently makes this technique compatible with high throughput and high resolution analyses [1–3]. The most recent important advancement was the introduction of the ultra-high performance liquid chromatography (UHPLC) technology in 2004, which combines columns packed with porous sub-2 μm particles with instruments possessing very low system void volumes that can withstand pressures up to 1000–1300 bar [4,5]. With appropriate column dimensions and flow rates, samples can be analyzed in only a few minutes or even seconds [6], whereas a plate count of up to 100,000 can also be attained in approximately 10 min [7]. Finally, analyses are obtained using robust instruments and analytical conditions. There are possible alternatives to UHPLC for improving the performance achieved in liquid chromatography. Among these alternatives, silica-based monoliths, which consist of a single rod of porous material with several unique features in terms of permeability and efficiency, could be interesting [8]. The second generation of silica-based monoliths, launched in 2011, provides performance equivalent to that of sub-2 μm particles at a reduced pressure drop [9], but they are scarcely used because of the limited number of available chemistries and dimensions. However, columns packed with sub-3 μm core-shell particles have recently been introduced on the market and can also be employed

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* Corresponding author. Tel.: +41 22 379 34 63; fax: +41 22 379 68 08.
E-mail address: davy.guillarme@unige.ch (D. Guillarme).

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to increase throughput and resolution [10–12]. Because the pressure generated by these superficially porous particles is two to three times lower compared to the fully porous sub-2 μm particles used in UHPLC, there is currently considerable interest in this technology, which is implemented in many academic and industrial laboratories.

In addition to changing the morphology and dimensions of the stationary phase, it is also possible to modify the properties of the mobile phase for improving the kinetic performance. It has already been demonstrated that elevated mobile phase temperatures (beyond 60 °C) in LC was a useful solution for decreasing the mobile phase viscosity, improving the diffusion coefficients and finally achieving faster separations at a reduced pressure drop [13–15]. However, this strategy could suffer from a possible thermal degradation of both the silica-based stationary phase and the analyzed compounds, specifically when using temperatures greater than 100 °C. Therefore, supercritical fluid chromatography (SFC) could be considered as a promising alternative. Since the early days of SFC, it has been demonstrated that supercritical fluids have a low viscosity, which results in fast diffusion of the compounds in the mobile phase, similarly to what can be observed in high temperature liquid chromatography, but without the risk of thermal degradation [16–18]. Therefore, the kinetic performance is improved while the generated backpressure remains reasonable [19,20]. Furthermore, this technology is considered to be a green approach; and using appropriate stationary phase chemistry, both non-polar [21,22] and polar ionizable compounds [22,23] and, even more recently, peptides [24] have been analyzed. However, the kinetic performance is often lower than expected because MeOH or other organic modifiers have to be added to the mobile phase to broaden the analysis range. In fact, the minimal height equivalent to a theoretical plate, $h_{\min}$, value remains identical irrespective of the %MeOH, as reported elsewhere [25,26], but the pressure drop is significantly higher because the viscosity is higher in the mobile phase, the mass transfer resistance is increased, and u_{opt} is reduced because the diffusion coefficients are lower in CO_2/MeOH vs. pure CO_2.

To avoid the kinetic limitations of conventional SFC in the presence of a mobile phase containing up to 20–30% MeOH, the new generation of stationary phases developed for LC (i.e., sub-3 μm core-shell technology or sub-2 μm fully porous particles) could be employed in SFC conditions. The proof of concept of this approach has very recently been demonstrated using the latest generation of core-shell columns [25] or columns packed with sub-2 μm particles [26,27]. The goal of the present contribution was to evaluate the use of columns packed with sub-2 μm particles in SFC conditions and to provide a more systematic comparison between the performance, advantages and limitations of both UHPLC and UHPSFC. For this purpose, both strategies were compared from a kinetic and thermodynamic point of view.

2. Experimental

2.1. Reagents and columns

LC–MS grade solvents (methanol, ethanol and heptane) were purchased from VWR (Radnor, PA, USA). Acetonitrile and formic acid (ULC–MS grade) were purchased from Biosolve BV (Valkenswaard, The Netherlands). Ultrapure water was obtained from a Milli-Q RG Purification unit from Millipore (Bedford, MA, USA). Free steroids (testosterone, 17-methyltestosterone, boldenone, stanozolol, 4-androsen-3,17-dione, dexamethasone, gestrinone and nandrolone) were generously gifted by the Swiss Laboratory for Doping Analysis (LAD) (Epalinges, Switzerland). Benzodiazepines and their derivatives (alprazolam, brotizolam, clonazepam, clorazepate, desmetylflunitrazepam, flunitrazepam, midazolam, nitrazepam, prazepam, triazolam and 7-aminoflunitrazepam) were provided by Lipomed AG (Arlesheim, Switzerland). Butylparaben, mefenamic acid, diclofenac, acetaminophen, chlorthalidone, indapamide, papaverine and noscapine were purchased from Sigma–Aldrich (Buchs, Switzerland).

SFC and UHPSFC columns: Viridis BEH (150 mm × 4.6 mm, 5 μm), Acquity UPC^2 BEH 2-Ethylpyridine (2EP) (100 mm × 3.0 mm, 3.5 μm), Acquity UPC^2 BEH, BEH 2-EP, CSH fluoro-phenyl (100 mm × 3.0 mm, 1.7 μm) and HSS C18SB (100 mm × 3.0 mm, 1.8 μm) were generous gifts from Waters (Milford, MA, USA). HPLC and UHPLC columns: XTerra RP18 (150 mm × 4.6 mm, 5 μm), XTerra RP18 (50 mm × 4.6 mm, 3.5 μm), Acquity BEH Shield RP18, BEH Phenyl, CSH fluoro-phenyl, BEH C18 (50 mm × 2.1 mm, 1.7 μm) and HSS C18SB (50 mm × 2.1 mm, 1.8 μm) were purchased from Waters.

2.2. Instrumentation and software

2.2.1. UHPLC system

The Waters Acquity UPLC system was equipped with a binary solvent delivery pump that possessed a 50 μL mixing chamber and was compatible with mobile phase flow rates up to 2 mL/min and pressures up to 1000 bar (upper pressure limit was 1000 bar at 1 mL/min and 600 bar at 2 mL/min), an autosampler that included a 5 μL loop, a column oven compatible with temperatures up to 90 °C and a UV detector that included a 0.5 μL flow-cell. The connection tube between the injector and the column inlet was 250 mm long (passive preheating included) and had an I.D. of 0.13 mm, and the capillary located between the column and detector was 150 mm long and had an I.D. of 0.1 mm. The extra-column volume of this instrument was estimated to be 14 μL, and the system dwell volume was 90 μL.

2.2.2. UHPSFC system

The Waters Acquity UPC^2 system (which stands for Acquity UltraPerformance Convergence Chromatography$^{\text{TM}}$) was equipped with a binary solvent delivery pump that possessed a 250 μL mixing chamber and was compatible with mobile phase flow rates up to 4 mL/min and pressures up to 414 bar (upper pressure limit was 414 bar at 3.25 mL/min and 293 bar at 4 mL/min), an autosampler that included a 10 μL loop, a column oven compatible with temperatures up to 90 °C, and a UV detector that included an 8 μL flow-cell and a backpressure regulator (BPR). The connection tube between the injector and column inlet was 600 mm long (active preheater included) and had an I.D. of 0.175 mm, and the capillary located between the column and detector was 600 mm long and had an I.D. of 0.175 mm. The extra-column volume of this instrument was estimated to be 59 μL, and the system dwell volume was 440 μL. Note that the majority of the experiments were conducted in the gradient mode, and consequently, the post-column dead volume (tubing from column outlet and UV detection and UV cell) was not critical for the separations.

2.2.3. Software

Data acquisition and control of the UHPLC systems were performed using the Waters Empower$^{\text{TM}}$ Pro 2 Software. The UHPSFC system was controlled with the Empower$^{\text{TM}}$ Pro 3 Software. Calculations were performed using MS Excel Software.

2.3. Procedure and methodology

Note that throughout the manuscript, the generic term "supercritical fluid" has been employed, but it would have been better, especially for the gradient mode separations, to indicate "subcritical fluid" separations. Indeed, when the percentage of MeOH in the