Journal of Chromatography A, 1403 (2015) 132–137

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Understanding and diminishing the extra-column band broadening effects in supercritical fluid chromatography

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ARTICLE INFO

Article history:
Received 31 March 2015
Received in revised form 7 May 2015
Accepted 7 May 2015
Available online 19 May 2015

Keywords:
Supercritical fluid chromatography
Detector cell
Extra-column band broadening
Extra-column volume
Sample solvent
Injection volume

ABSTRACT

Supercritical fluid chromatography, where a low-viscosity mobile phase such as carbon dioxide is used, proves to be an excellent technique for fast and efficient separations, especially when sub-2 μm particles are used. However, to achieve high velocities when using these small particles, and in order to stay within the flow rate range of current SFC-instruments, narrow columns (e.g. 2.1 mm ID) must be used. Unfortunately, state-of-the-art instrumentation is limiting the full separation power of these narrower columns due to significant extra-column band broadening effects. The present work identifies and quantifies the different contributions to extra-column band broadening in SFC such as the influence of the sample solvent, injection volume, extra-column volumes and detector cell volume/design. When matching the sample solvent to the mobile phase in terms of elution strength and polarity (e.g. using hexane/ethanol/isopropanol 85/10/5 vol%) and lowering the injection volume to 0.4 μL, the plate count can be increased from 7600 to 21,300 for a low-retaining compound (k' = 2.3) on a 2.1 mm × 150 mm column (packed with 1.8 μm particles). The application of a water/acetonitrile mixture as sample solvent was also investigated. It was found that when the volumetric ratio of water/acetonitrile was optimized, only a slightly lower plate count was measured compared to the hexane-based solvent when minimizing injection and extra-column volume. This confirms earlier results that water/acetonitrile can be used if water-soluble samples are considered or when a less volatile solvent is preferred. Minimizing the ID of the connection capillaries from 250 to 65 μm, however, gives no further improvement in obtained efficiency for early-eluting compounds when a standard system configuration with optimized sample solvent was used. When switching to a state-of-the-art detector design with reduced (dispersion) volume (1.7–0.6 μL), an increase in plate count is observed (from 11,000 to 14,000 plates on a 2.1 mm × 100 mm column with 1.8 μm particles for k' = 3) even when 250 μm tubing was used. Using this detector cell and decreasing the ID of the tubing from 250 to 120 μm resulted in an additional increase to 17,300 plates. Further decreasing the tubing ID (e.g. 65 μm) appeared to have no observable influence on the obtained plate count.

1. Introduction

In order to achieve fast and efficient separations in chromatography, it is important to decrease the resistance for mass transfer. Among several approaches, using a low-viscosity fluid, such as fluidic CO_2, results in very high diffusion coefficients and thus allows the use of higher flow rates without significant efficiency loss [1]. As a result, very fast and efficient separations can be performed in supercritical fluid chromatography (SFC) [2–7]. For example, a plate count of 182,000 plates can be achieved for a void time of 5.8 min when the pressure limitation of state-of-the-art instrumentation (600 bar) is considered [8].

Although this makes carbon dioxide an attractive solvent for chromatography, working with a highly compressible mobile phase has some drawbacks of its own. First of all, a back pressure above the critical pressure (72 bar) is required to avoid the formation of gas bubbles in the detector cell. Typically, even higher back pressures are used (120–150 bar) to operate in a region sufficiently far from the critical point, i.e. far from the region of high compressibility of the mobile phase. Otherwise, the large changes in mobile phase density can cause extensive decompression cooling which results in a loss in performance and significant variation in velocity and retention along the column occurs due to the large variation in density [9–13]. Another consequence is the inability to

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http://dx.doi.org/10.1016/j.chroma.2015.05.017
0021-9673/

precisely match the sample solvent with the mobile phase, as this would require the sample to be compressed in a CO_2-based mobile phase. Depending on the column ID and the injected volume, this mismatch between sample solvent and mobile phase may lead to significant extra-column band broadening especially in preparative scale separations [16]. Several studies highlighted the effect of sample solvents on performance in SFC but even with this knowledge, issues with extra-column band broadening still remain for narrow columns. In general it was found that apolar solvents such as heptane allow to minimize peak distortion, whereas another study found that acetonitrile in combination with water could be used if water-soluble samples are considered [14,15,17,18].

A recent investigation of Perrenoud et al. showed that, due to the significant extra-column variance in SFC, the optimal column geometry would be a 3 mm ID column in the case of small particles and short columns. This ID would be a perfect trade-off between minimizing extra-column band broadening effects and still being able to achieve sufficiently high linear velocities for e.g. sub-2 μm particles [6]. A previous study about speed-resolution limits in SFC also illustrated important extra-column band broadening effects for early-eluting compounds on short columns with a 2.1 mm ID format, which were largely resolved when using very long columns (e.g. 500 mm) [8].

Nonetheless, the origin and relative contributions of extra-column band broadening in SFC are not entirely understood or quantified even though it follows the same basic principles as e.g. in liquid chromatography (LC). The potential sources are:

- Sample solvent: even though the use of hexane/ethanol (EtOH)/isopropanol (IPA), where IPA is added to allow better mixing of EtOH and hexane [6], as a sample solvent provides a reasonable viscosity and retention match with the CO_2/methanol (MeOH) mobile phase, it is not perfect and may still lead to a distortion of the sample band at the column inlet. In addition, a water (H_2O)/acetonitrile (ACN) solvent will also be assessed since it may be of practical use when testing water-soluble samples [17].
- Injection volume: SFC-injectors generally work via the fixed-loop principle where the injection loop is generally large (5 or 10 μL). If partial-loop injection is considered, the rest of the loop is generally filled with the mobile phase co-solvent (or another wash solvent). At the moment of injection, a large amount of mismatching solvent hence enters the column with the sample, which may lead to peak distortion.
- Extra-column volumes: in SFC, generally long tubing with higher ID's are used (170 μm instead of the more common 120 or even 75 μm in LC), which could lead to severe extra-column band broadening. In addition, the geometry of the extra-column volumes (e.g. detector design) may also lead to an additional contribution due to the presence of stagnant zones.

The present work investigates and quantifies the relative contributions of these potential band broadening sources for current state-of-the-art SFC instrumentation and explores some new ways to reduce efficiency losses for low-retained compounds.

2. Experimental

2.1. Column, tubing and chemicals

Methanol, hexane and isopropanol (LC-MS grade) were purchased from Biosolve (Valkenswaard, Netherlands), CO_2 was purchased from Air Liquide (Paris, France). Test components such as testosterone, chlorthalidone, bendroflumethiazide, altizide and β-estradiol were kindly provided by Deirdre Cabooter (Laboratory of Pharmaceutical Analysis, KU Leuven, Belgium). The samples were dissolved in a mixture of ethanol (EtOH), isopropanol (IPA) and hexane (except for the assessment of the water/acetonitrile solvent). The IPA was added to allow mixing of EtOH with hexane [6]. In order to inject different sample volumes, a 20 cm fused-silica 50 μm capillary, a 7 cm, 120 μm and a 11 cm, 170 μm stainless steel capillary were used, to achieve, respectively, a 0.4, 0.8 and 2.5 μL injection volume (instead of the 5 μL sample loop intended for the standard configuration of the instrument). All the reported extra-column volumes are calculated, based on the nominal dimensions given by the manufacturers. These nominal dimensions were evaluated by measuring the total extra-column volume under LC conditions and a deviation of maximally 15% was found.

2.2. Instrumentation and conditions

The SFC-system used in the study was a modified Agilent G4301A-based SFC system in combination with a thermostatted column compartment, autosampler used in full-loop mode and two flow cells with a dispersion volume of 1.7 μL (DAD G1315C with a G1314-60082 flow cell) and 0.6 μL (Agilent 1290 Infinity DAD G4212A with a G4212-60038 flow cell). Zorbax HILIC RRHD columns (2.1 mm ID, 150 mm and 100 mm, 1.8 μm fully porous particles) were used in the current study. The columns were kindly provided by Xiaoli Wang (Agilent Technologies, Little Falls, USA). The oven temperature was set at 40 °C, 8 v% methanol as modifier was used and the back pressure was set at 150 bar, unless otherwise specified. The detector was set at an acquisition rate of 100 Hz and a wavelength of 230 nm. Due to the high acquisition rate, the observed peak widths and variances were not affected, even for very the narrow, early-eluting peaks. The shown data are average values of 3 repeats and a maximum relative standard deviation for the plate count of 6% was found.

3. Results and discussion

3.1. Possibilities with current state-of-the-art SFC instrumentation

The first goal is to investigate the possibilities to reduce peak broadening in state-of-the-art SFC systems. For this purpose we have

- varied the sample composition.
- varied the injection volume by using 0.4 and 2.5 μL loops.
- varied pre- and post-column volumes by choosing smaller ID tubing or switching the preheater (e.g. 1.6 μL instead of 3 μL preheater intended for the standard configuration of the used instrument).

3.1.1. Sample solvent and extra-column volume

To study the influence of extra-column volume, only the volume before the column (extra-precolumn volume) was changed by: changing the preheater (1.6 or 3 μL) and the connection capillary from injector to preheater (120 or 170 μm). This resulted in two different extra-precolumn volumes of 4.4 μL (20 cm, 120 μm connection capillary from injector to preheater, 1.6 μL preheater) and 7.4 μL (17 cm, 170 μm connection capillary, 3 μL preheater). The same 5 cm long 120 μm connection capillary from preheater to column was kept for both configurations.

The injection volume was varied by using either the 0.4 or the 2.5 μL loop in the overfill mode. Whereas commercial SFC-systems are typically supplied with a 5 μL-loop, smaller loops can be used but it is not recommended on a regular basis as it may lead to system errors during the washing or filling phase due to an increased