



Effect of reference conditions on flow rate, modifier fraction and retention in supercritical fluid chromatography



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ABSTRACT

When using compressible mobile phases such as fluidic CO₂, the density, the volumetric flow rates and volumetric fractions are pressure dependent. The pressure and temperature definition of these volumetric parameters (referred to as the reference conditions) may alter between systems, manufacturers and operating conditions. A supercritical fluid chromatography system was modified to operate in two modes with different definition of the eluent delivery parameters, referred to as fixed and variable mode. For the variable mode, the volumetric parameters are defined with reference to the pump operating pressure and actual pump head temperature. These conditions may vary when, e.g. changing the column length, permeability, flow rate, etc. and are thus variable reference conditions. For the fixed mode, the reference conditions were set at 150 bar and 30 °C, resulting in a mass flow rate and mass fraction of modifier definition which is independent of the operation conditions. For the variable mode, the mass flow rate of carbon dioxide increases with system pump operating pressure, decreasing the fraction of modifier. Comparing the void times and retention factor shows that the deviation between the two modes is almost independent of modifier percentage, but depends on the operating pressure. Recalculating the set volumetric fraction of modifier to the mass fraction results in the same retention behaviour for both modes. This shows that retention in SFC can be best modelled using the mass fraction of modifier. The fixed mode also simplifies method scaling as it only requires matching average column pressure.

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1. Introduction

Design of chromatographic pumps that deliver an accurate and repeatable flow rate is of high importance to avoid poor retention time precision and to obtain reproducible peak areas. Whereas typical operating pressures in classical high-performance liquid chromatography (HPLC) systems allowed to assume that the density of the mobile phase is constant, this is no longer the case for so-called ultra-high-performance liquid chromatography that operates at pressures up to 1000–1500 bar. Additionally, this compressibility depends on the employed mobile phase and is larger for most organic solvents than for water (H₂O). For example, the density of H₂O changes by 6% and up to 12% for methanol (MeOH) when going from 1 to 1500 bar at 30 °C [1].

In addition, the heat generated inside the liquid during compression decreases its density and makes correct metering of the flow rate more complicated. A detailed discussion of the

operating mode of modern UHPLC pumps was recently given by De Vos et al. [2]. In brief, these effects are compensated either by estimating solvent compressibility and their thermal capacity or by an iterative correction algorithm using the monitored pressure profile [2]. When using a highly compressible mobile phase, such as fluidic CO₂, both the effect on the total mass flow rate and the difference in compressibility of the mobile phase constituents are expected to be even more pronounced.

In 2013, Tarafder et al. pointed out the importance of correctly measured mass flow rates to describe the conditions for separations using compressed, fluidic CO₂ as main mobile phase constituent (often referred to as supercritical fluid chromatography or SFC) [4]. Since the mass flow rate is the only flow parameter which does not vary along the column, at least under any steady state conditions, it is reasonable to strive for accuracy of its value. In order to measure the mass flow rate on-line, several authors used a Coriolis flow meter to achieve more reproducible separations for example in terms of retention times [5–7]. Tarafder and Guiochon showed the possibilities of such a device for continuous diagnosis of the correct operation of the SFC instrument and for monitoring the carbon dioxide supply [5]. The group of Fornstedt pointed

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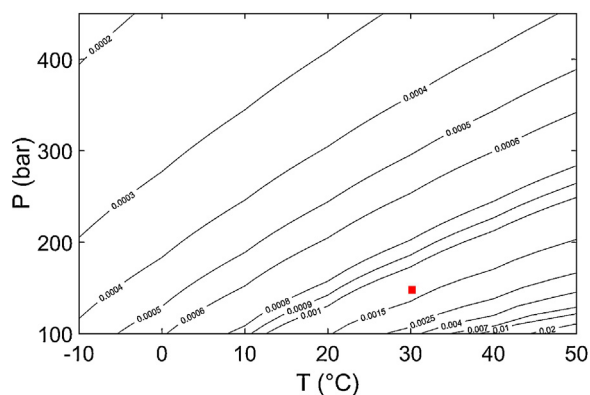


Fig. 1. Isothermal compressibility (bar^{-1}) as a function of pressure and temperature for neat CO_2 . The \blacksquare denotes the reference conditions for the fixed mode.

out that the mass flow rate is a crucial parameter for scaling from an analytical to a preparative scale [6,7], since most analytical systems are volume flow-controlled whereas preparative systems are mainly mass flow-controlled. In addition, the group recently pointed out the significant difference between the set (i.e. the value set on the instrument) and actual or measured values of flow rate and volumetric fraction of modifier and its impact on the construction of surface excess adsorption isotherms of methanol on a diol silica adsorbent, which illustrates the importance of not only reproducibility but primary of accuracy of the eluent flow and composition [8]. Besides the methanol fraction, the average pressure (or average density) was found to be the most important parameter [7].

In Fig. 1, the isothermal compressibility is given as a function of pressure and temperature for neat CO_2 . This plot represents similar data as the so-called isopycnic plots (lines of constant density) but is more practical for the current discussion [9–11]. Under laboratory conditions (30°C), the compressibility of fluidic CO_2 is equal to 0.001 bar^{-1} at 150 bar, which is roughly 10 times higher than for an organic solvent such as MeOH [1,3]. As a result, the delivered mass flow rate will change significantly with e.g. pump operating pressure (P_{pump}) when the same volumetric flow rate is delivered. One way to mitigate this issue is to cool the pump head down to or below 0°C to operate CO_2 way below its critical temperature, as its isothermal compressibility is then significantly lower. However, the compressibility of fluidic CO_2 is still three times higher than for MeOH, even at -10°C and 150 bar. Knowledge of the delivered mass flow rate is thus crucial for reliable and predictable SFC separations. However, the currently available flow meters are typically expensive, may have a slow response and a limited pressure rating and are thus impractical for routine use.

In the current contribution, the effect of metering flow rates at different reference conditions is investigated. For the fixed mode, the flow rate and volumetric fraction of modifier set on the instrument will always correspond to a fixed pressure (150 bar) and temperature (30°C), whereas in the variable mode the volumetric flow rate and modifier fraction is delivered at the pump's operating pressure (see Section 2). The resulting mass flows, mass fractions and retention times are compared for these different operating modes.

2. Experimental

2.1. Column, tubing and chemicals

Methanol (LC-MS grade) was purchased from Biosolve (Valkenswaard, Netherlands), CO_2 was purchased from Air Liquide (Paris, France). Test components such as aspirine, progesteron, testosterone, β -estradiol, bendroflumethiazide and chlorthalidone

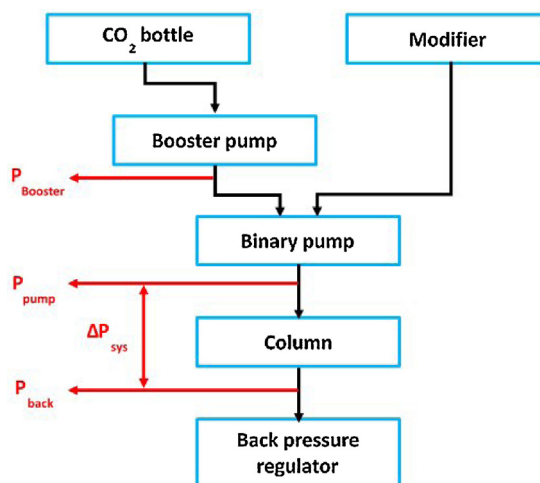


Fig. 2. Schematic overview of the SFC system. The following pressures are defined: P_{pump} (binary pump pressure), P_{back} (back pressure regulator pressure), P_{booster} (booster pressure) and ΔP_{sys} (system pressure drop).

were purchased from Sigma-Aldrich (Diegem, Belgium). The samples were dissolved in a mixture of ethanol (EtOH), isopropanol (IPA) and hexane of 20/10/70 vol% at a concentration of $100 \mu\text{g}/\text{mL}$ [12,13]. A $3 \times 150 \text{ mm}$ HILIC Poroshell column (packed with $2.7 \mu\text{m}$ superficially-porous particles) was used for all the measurements.

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, an autosampler used in full-loop mode and a flow cell with a volume of $1.7 \mu\text{L}$ (DAD G1315C with a G1314-60082 flow cell).

A schematic overview of the system is given in Fig. 2. Gaseous CO_2 is delivered to the booster pump, which compresses the gas to a liquid. The pressure to which the booster pump compresses the gas is defined as P_{booster} . The binary pump (or metering pump) further compresses the fluidic CO_2 towards the necessary pressure to deliver a certain set flow rate. This pressure is defined as P_{pump} . The definition of the set flow rate and volumetric fractions is dependent on the operating mode of the system. The system allows to operate in two modes:

- **Variable mode:** The volumetric flow rate set on the instrument corresponds to the pump conditions (nearly lab temperature and pump operating pressure). This results in a constant (equal to the set value) volumetric flow rate at the column inlet but a variation of the mass flow rate when the system pressure changes as the density of the pumped CO_2 will change. This mode is similar to that of a commercially available Agilent 1260 Infinity Analytical SFC system.
- **Fixed mode:** The set volumetric CO_2 flow rate is corresponding to fixed reference conditions (e.g. 150 bar and 30°C). This means that if the delivered volumetric flow rate were to be measured at 150 bar and 30°C , it would correspond to the set one. In other words, the set volumetric flow multiplied with the density of CO_2 at 30°C and 150 bar always corresponds to the delivered mass flow rate. This results in a pressure and temperature independent mass flow rate, but a varying volumetric flow rate at the column inlet when the pump operating pressure changes.

The set modifier flow rate is always defined and delivered at atmospheric pressure and lab temperature. The column oven temperature was set at 40°C in all experiments. The temperature of

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