Journal of Chromatography A, 1339 (2014) 168–173

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Determination of the average volumetric flow rate in supercritical fluid chromatography

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ARTICLE INFO

Article history:
Received 27 October 2013
Received in revised form 16 February 2014
Accepted 27 February 2014
Available online 12 March 2014

Keywords:
Average volumetric flow rate
Nitrous-oxide
Hold-up volume
Supercritical fluid chromatography

ABSTRACT

This work reviews and discusses controversies and errors made in the determination of the average volumetric flow rate of a compressible mobile phase forced to flow through a chromatographic column. Proper estimates of the volumetric flow rate, which obviously changes along the column, are keys to understanding the retention mechanism that takes place inside the column and to achieve repeatable and reproducible separations. Each step of the calculation process will be discussed in detail, including how to estimate the variations of the pressure and the temperature along the column. The determination of the average volumetric flow rate requires the knowledge of the average density of the mobile phase and of its mass flow rate. The calculations were carried out under various experimental conditions, including different column temperatures and inlet pressures. The estimated values of the volumetric flow rate are validated by the conversion of the retention times to the retention volumes of nitrous oxide peaks, which is valid since this compound is assumed to be non retained, which makes it a hold-up time marker.

1. Introduction

The determination of the true volumetric flow rate of the mobile phase along the instrument channels and column is critical in any chromatographic applications. Accurate knowledge of this flow rate is necessary for a proper understanding of the retention mechanisms and for the planning of any analytical or preparative scale separation [1,2]. This task is as necessary in supercritical fluid chromatography (SFC) as it is in HPLC but it is made more difficult and complex by the properties of the mobile phase [3–5]. Mobile phases used in SFC are far more compressible than conventional solvents used in HPLC. As a consequence the mobile phase density varies along the column and so does its volumetric flow rate. The formation of a density gradient along the column has several consequences on the chromatographic process because equilibrium constants of solutes between stationary and mobile phases depend on the local pressure. Due to the variations of the volumetric flow rate, the adsorption equilibrium constants, the retention factors, and the diffusivities of solutes change along the column [6].

In most cases, the volumetric flow rate set in the instrument software does not provide the actual average value of the volumetric flow rate through the column. Software used to operate SFC instruments use the set volumetric flow rate to actuate and control the back-and-forth movements of the pump piston. Due to the mobile phase compressibility, the set volumetric flow rate provides the real value of the average volumetric flow rate of the mobile phase only in some cases, when the pressure drop along the column is small [7,8].

If SFC separations are carried out with a column packed with small diameter particles – e.g., smaller than 3 μm – the pressure drop along this column reaches a magnitude that causes a non-negligible change of the mobile phase density along the column [5]. As the mobile phase density drops, its volumetric flow rate increases significantly, making more necessary and more difficult the determination of a correct estimate of the local volumetric flow rate. However, the constant trend of analysts to perform faster and more efficient separations makes it nearly impossible to avoid the use of columns packed with fine particles.

Due to the complexity of the design of modern instruments, static methods cannot be used to determine the column hold-up volumes, another necessary parameter. Dynamic methods must be used but they require the introduction of a hold-up time marker compound [9]. The determination of this crucial system property is at the cornerstone of a proper understanding of the retention mechanisms involved [10,11].

The goal of this paper is to examine how to estimate the average volumetric flow rate in chromatographic systems operated with compressible mobile phases. In most cases, the volumetric flow rate is set on the instrument control software to a value that rarely

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http://dx.doi.org/10.1016/j.chroma.2014.02.078
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reflects the real value inside the column. Due to the mobile phase compressibility, a density gradient forms along the column resulting in an actual local volumetric flow rate that increases from the column inlet to its outlet.

To obtain an estimate of the influence of the average volumetric flow rate on the density profile along the column requires the knowledge of important experimental parameters, the mass flow rate of the SFC fluid and the temperature and pressure drops along the column. An accurate knowledge of the local temperature and pressure of the instrument in any point permits an estimate of the average mobile phase density along the column or along the instrument. Knowing the average mobile phase density and its mass flow rate should permit highly accurate estimates of the average volumetric flow rate.

The validation of the estimated average volumetric flow rates obtained by independent measurements requires the use of a hold-up time marker. Using the average volumetric flow rate, the hold-up time of a compound can be converted into the column hold-up volume. Comparison of this column hold-up volume with a value determined by a static method may validate the average volumetric flow rate obtained under different experimental conditions.

An accurate estimate of the volumetric flow rate that is related to the density drop along the column is critical for any thermodynamic study and plays an important role in the ability to perform repeatable and reproducible measurements. Method transfer between instruments also requires knowledge of the average volumetric flow rate in each instrument. Because different instruments are operated under different conditions, e.g., pump head temperature (that affects the density, hence the mass flow rate at constant inlet volumetric flow rate), the same volumetric flow rate set on two different instruments will not provide the same experimental conditions if the mass flow rates and the average densities of the mobile phase, i.e., the average volumetric flow rate are different. Finally, any optimization or design process of large scale separations is impossible without knowledge of the average volumetric flow rate.

2. Materials and reagents

The measurements were carried out using a Waters UPC^2 supercritical fluid chromatograph (Milford, MA). The instrument includes a binary head pump (2 °C), with an accumulator (13 °C) for carbon dioxide and an organic modifier pump, an automated injector with a 10 μL sample loop, a column thermostat, a back pressure regulator, a diode array UV/VIS detector and a data station.

A 4.6 mm × 100 mm Phenomenex (Torrence, CA, USA) Kinetex C_{18} column packed with 2.6 μm core-shell particles was used in this study. Carbon dioxide was purchased from Airgas (Knoxville, TN, USA), nitrous-oxide from Sigma–Aldrich (St. Louis, MO, USA), and methanol from Fisher Scientific (Fair Lawn, NJ, USA).

The measurements were carried out at column oven temperatures of 10, 20, 30, 40, 50 and 60 °C. The flow rates set up on the carbon dioxide pump were 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 cm^3/min. All the measurements were carried out with a constant 104 bar back pressure. The UV-detector signal was recorded between 190 and 280 nm. The most suitable wavelength to detect nitrous oxide was at 202 nm.

The temperatures of the mobile phase at the column inlet and outlet were measured using insulated temperature sensors. The mass flow rate of the CO_2 stream was measured after the column at room temperature with a mini CORI-FLOW instrument from Bronkhorst High-Tech B.V. (Ruurlo, NL), Model No. M13-ABD-11-0-S, Serial No. B11200776A. This model provides an accuracy of ±(0.2% of the read value +0.5 g/h). The mobile phase densities were calculated using the NIST REFPROP database.

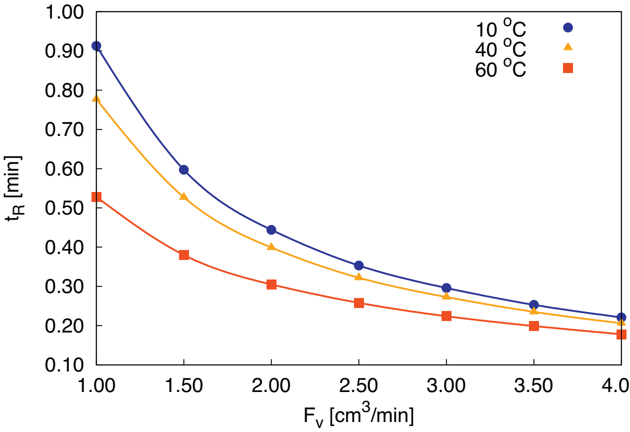

Fig. 1. Retention times of nitrous-oxide at different column temperature and set volumetric flow rate of carbon dioxide. The volumetric flow rate, F_V was set by the software of the instrument.

3. Results and discussion

In all measurements, a plug of 0.1 μL nitrous oxide dissolved in pure methanol was injected into the stream of pure carbon dioxide. Nitrous oxide was bubbled into pure methanol for a period of approximately 1 min. Nitrous oxide does not interact with the stationary phase and gives a well resolved peak from the solvent peak of methanol, so its retention time is the hold-up time of the instrument, with no systematic error and a small random error in the same range as are the applied experimental parameters [9]. The retention times of nitrous-oxide were corrected with the extra-column contributions in all cases.

The first part of this section describes the methodology used to estimate the average volumetric flow rate. To test the accuracy of the values calculated, experiments were carried out under rather extreme conditions, under which the mobile phase compressibility is high. The average volumetric flow rates obtained were used to convert the hold-up times of nitrous oxide into hold-up volume data. These data were compared with those determined by a static method.

This section ends with a study of the differences between the set volumetric flow rate and the average volumetric flow rate that arise when the back pressure is changed or an organic modifier is mixed to the mobile phase.

3.1. *The retention time pattern of nitrous oxide peaks*

The variations of the retention times of nitrous oxide peaks with the column temperature and the set volumetric flow rate of the mobile phase are shown in Fig. 1, in which two trends are observed. First, the retention time of nitrous oxide decreases with increasing volumetric flow rate of carbon dioxide. Second, this retention time decreases with increasing column temperature. The relative importance of the retention times variations with increasing flow rate increases with increasing temperature beyond 40 °C. This suggests that the volumetric flow rate set on the delivery pump may markedly differ from the actual rate in the investigated temperature range.

3.2. *Pressure and temperature changes along the column*

Our calculations assumed a linear decrease of the pressure and the temperature along the column. The column inlet pressure was the pump-head pressure while the outlet pressure was the set back-pressure. The temperatures of the mobile phase at the column inlet