Maximizing performance in supercritical fluid chromatography using low-density mobile phases

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Abstract

The performance of a 3.0×150 mm column packed with $1.8 \mu\text{m}$ fully porous HSS-SB- C_{18} particles was investigated in supercritical fluid chromatography (SFC) with low-density, highly expansible carbon dioxide. These conditions are selected for the analysis of semi-volatile compounds. Elevated temperatures ($>100^\circ\text{C}$) were then combined with low column back pressures (< 100 bar). In this work, the inlet temperature of pure carbon dioxide was set at 107°C , the active back pressure regulator (ABPR) pressure was fixed at 100 bar, and the flow rate was set at 2.1 mL/min at 12°C (liquefied carbon dioxide) and at an inlet column pressure close to 300 bar. Nine *n*-alkylbenzenes (from benzene to octadecylbenzene) were injected under linear (no sample overload) conditions. The severe steepness of the temperature gradients across the column diameter were predicted from a simplified heat transfer model. Such conditions dramatically lower the column performance by affecting the symmetry of the peak shape. In order to cope with this problem, three different approaches were experimentally tested. They include 1) the decoupling and the proper selection of the inlet eluent temperature with respect to the oven temperature, 2) the partial thermal insulation of the column using polyethylene aerogel, and 3) the application of a high vacuum (10^{-5} Torr provided by a turbo-molecular pump) in a housing chamber surrounding the whole column body.

The results reveal that 1) the column efficiency can be maximized by properly selecting the difference between the eluent and the oven temperatures, 2) the mere wrapping of the

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column with an excellent insulating material is insufficient to fully eliminate heat exchanges by conduction and the undesirable radial density gradients across the column i.d., and 3) the complete thermal insulation of the SFC column under high vacuum allows to maximize the column efficiency by maintaining the integrity of the peak shape.

Keywords: Supercritical fluid chromatography; low-density carbon dioxide; column efficiency; vacuum technology; decoupling between inlet eluent temperature and oven temperature; insulating material.

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1 Introduction

Thermal phenomena that may affect resolution performance in both LC and SFC result from the significant amount of heat exchanged between the column walls and its surrounding environment (either ambient lab air or oven still-air under standard operating conditions). This heat flux is driven by the temperature difference between the column and its external environment. In LC, it originates from the dissipation of viscous heat inside the packed beds when columns are run at high flow rates and high pressure drops ¹⁻⁶. In SFC, the same temperature difference is caused by the cooling of the mobile phase during its decompression ^{7,8} and/or by the possible mismatch between the column temperature (wall) and the external lab or oven temperature. In all cases, the stationary radial temperature gradients ⁹ that form across the column diameter may dramatically affect column performance ^{5,6,10-12} due to the transverse changes in chromatographic properties . The two most critical properties are the local flow velocity and the local equilibrium constant of the analytes between the stationary and mobile phases. They both determine the local migration velocity of the analyte and the shape of the chromatographic band observed at the column outlet ^{8,12-16}. This may lead to severe band distortion that are no longer acceptable for the resolution of complex mixtures. Yet, the band deformation is smoothed to some extent by the intensity of the transverse dispersion coefficient of the analytes across the packed bed ¹⁷. Transverse dispersion tends to even out the radial concentration gradients ¹⁸ but, for usual LC and SFC chromatographic speeds, it is not intense enough to maintain acceptable peak shapes ^{8,13,16}. Therefore, whether heat is absorbed or released by the chromatographic column, experimental strategies aiming at minimizing the intensity of the steady state heat flux or at reversing its direction (from the column to its surrounding to from the surroundings to the column) are needed. For instance, the application of a high vacuum around a vHPLC column was successfully applied. The resolution performance was improved by 40% at the fastest affordable speed (15 000 psi) with respect to standard still-air operating conditions ^{19,20}.

SFC performance is particularly affected by thermal effects when the mobile phase selected by the user is highly expansible, e.g., when its Joule-Thomson coefficient, which measures the rate of the eluent cooling per unit of pressure drop along the column ⁴, becomes excessively large. In

practice, this concerns mobile phase containing a small amount ($< 5\%$ in volume) of polar solvents (methanol, ethanol, isopropanol,...), low ABPR pressures (< 125 bar), and high inlet temperatures (> 70 °C)²¹. Such extreme operating conditions are often required for the analysis of semi-volatile apolar compounds (terpenes, gasoline, fragrances,...) that cannot be sufficiently retained by any available SFC stationary phase with different surface chemistries (diol, C₁₈, 2-picolylamine, diethylamine, ethylpyridine, ...) under standard conditions. It is important to recall that any other SFC conditions involving high organic solvent concentrations, high ABPR pressures, or ambient and moderately high temperatures are always providing to the chromatographer the maximum column performance²². However, the advantages of using low-density SFC mobile phases are multiple : 1) the retention increase of semi-volatile compounds allows their separation, 2) faster analyses than vHPLC or micro-HPLC analyses can be achieved^{23,24}, 3) the co-elution of these analytes with most sample solvents can be avoided when using flame ionization detection²⁴, and 4) no headspace GC analysis is needed since they can be separated in the presence of heavier compounds by applying a gradient of either organic modifier, flow rate or column back pressure²⁴. The only drawback could be the potential band stretching due to the overlap between the sample band and that of the strong sample solvent²⁵⁻²⁷ and to the above-mentioned thermal effects.

In this work, three different strategies are achieved in the lab to minimize these thermal effects and to improve the peak shape of semi-volatiles compounds in SFC when low-density carbon dioxide is used. They include the independent control of the inlet eluent temperature and of the oven temperature, wrapping the SFC column with an insulating material (polyethylene foam), and the application of a high-vacuum (10^{-5} Torr) in an air-housing chamber enveloping the chromatographic column. 100% carbon dioxide, an inlet temperature of 107 °C, and an ABPR pressure of 100 bar are selected. A 3.0 mm $\times$ 150 mm column packed with 1.8 μ m fully porous HSS-SB-C₁₈ particles (the column pressure drop is around 200 bar) is selected for high performance SFC. For such experimental conditions, the Joule-Thomson coefficient increases from 0.1 to 0.6 K/bar (NIST REFPROP data) from the column inlet to the column outlet and the adiabatic cooling of the mobile phase is very intense approaching 50 °C. The semi-volatile compounds are seven *n*-alkylbenzenes including benzene (C₀), ethylbenzene (C₂), butylbenzene (C₄), hexylbenzene (C₆), **octylbenzene**

102 (C₈), decylbenzene (C₁₀), and dodecylbenzene (C₁₂). The performance of each proposed experi-
103 mental strategy is reported in terms of improvement of the peak shapes and efficiency of the SFC
104 column when operated under such challenging conditions.

2 Theory

In this section, a simplified but very practical model of heat transfer is proposed in order to calculate and predict the radial temperature profiles across a SFC column operated with a low-density mobile phase. The necessary physico-chemical data required for the calculation of these temperature profiles from the center of the chromatographic bed ($r = 0$) to the external ambient environment ($r = \infty$) are given for pure carbon dioxide. The chromatographic column (stainless steel tube, inner radius $r_c=3.00$ mm, external radius $r_e=8.00$ mm) is 15 cm long and packed with 1.8 μm High Strength Silica (HSS) Stable Bond (SB) C_{18} particles. It is placed horizontally at the center of a cylindrical housing chamber (stainless steel tube, inner radius $R_i=3$ cm, external radius $R_e=3.84$ cm). The space in between the two coaxial stainless steel tubes is filled with air at either atmospheric pressure (760 Torr, thermal conductivity $\lambda_{air}=0.026$ W/m-K) or at reduced air pressure (10^{-5} Torr) or with an insulating aerogel material (Tubolit polyethylene pipe, thermal conductivity $\lambda_{PE}=0.036$ W/m-K). In all three cases, the stainless steel tube of the housing chamber is placed in still-air condition at lab temperature ($T=297$ K). A constant flow rate of $F_v=2.1$ mL/min (100% carbon dioxide pumped at 12 °C and close to 300 bar pressure when the ABPR pressure is set at 100 bar) generates a constant pressure drop $\Delta P=200$ bar along the 3.0 mm $\times$ 150 mm long column. The overall amount, P_{heat} (Watt), of viscous and expansion heat dissipated per unit time along the column is estimated by ^{1, 6, 11, 12, 14, 28, 29}:

$$P_{heat} = (1 + \langle \alpha T \rangle) \langle F_v \rangle \Delta P \quad (1)$$

$$= (1 + \langle \alpha T \rangle) \frac{180(1 - \epsilon_e)^2 \langle \eta \rangle L}{\epsilon_e^3 d_p^2} \frac{\langle F_v \rangle^2}{\pi r_c^2} \quad (2)$$

where $\langle F_v \rangle$, $\langle \eta \rangle$, and $\langle \alpha T \rangle$ are the average flow rate, viscosity, and product of the temperature by the thermal expansion coefficient, respectively, of the mobile phase along the column. $\epsilon_e=0.385$ is the interstitial porosity of the packed bed and $d_p=1.8$ μm is the average particle diameter. For pure carbon dioxide (no organic solvent), $T_{inlet}=380$ K, $T_{outlet}=330$ K, $P_{inlet}=300$ bar, and $P_{outlet}=100$ bar, then, $\langle F_v \rangle=3.8$ mL/min, $\langle \eta \rangle=0.038$ cP and $\langle \alpha T \rangle=-5.07$ (data from

NIST REFPROP software).

Assume that the column is placed in a strict adiabatic environment, then, the radial flux of dissipated heat is zero, the temperature profile across the column diameter is uniform, and the temperature difference, $\Delta_L T$, between the outlet and inlet of the chromatographic bed is ^{1,11,30}:

$$\Delta_L T = (1 + \langle \alpha T \rangle) \frac{\Delta P}{\langle C_p \rangle} < 0 \quad (3)$$

where $\langle C_p \rangle = 1.44 \times 10^6 \text{ J/m}^3/\text{K}$ is the average heat capacity of pure carbon dioxide along the column. Accordingly, $\Delta_L T$ is expected to be around 56 °C. In fact, $\Delta_L T$ was measured at exactly 52 °C under quasi-adiabatic conditions (see later in the results and discussion section), so, the proposed construction of the heat transfer model is fully consistent with the observation and relevant for our purpose.

Assume the heat power is entirely evacuated across the column diameter by maintaining constant and uniform the column wall temperature (water bath ¹¹ of forced air convection ²⁰), then $\Delta_L T = 0$ and the temperature difference, $\Delta_R T$, between the center and the wall of the chromatographic bed is estimated by ¹¹:

$$\Delta_R T = -(1 + \langle \alpha T \rangle) \frac{\langle F_v \rangle \Delta P}{4\pi L \langle \lambda_{eff} \rangle} > 0 \quad (4)$$

where $\langle \lambda_{eff} \rangle = 0.26 \text{ W/m-K}$ is the average effective thermal conductivity of the packed bed when it is immersed in the supercritical fluid mobile phase ¹⁹. Accordingly, for fully developed radial temperature gradients ^{2,3}, the largest amplitude of the radial temperature gradient across the 3.0 mm i.d. column is close to 10 K.

Note that, in practice, none of the above scenarios is actually met. A fraction, f , of the overall heat given by Eq. 1 is also lost to the cooler external environment (the ambient lab air temperature is $T = 297 \text{ K}$). The intensity of f depends on the quality of the thermal insulation of the column and on the applied flow velocity. $f = 0$ when the column is perfectly insulated. f is maximum when the temperature of the column wall is maintained constant at ambient or cooler temperature. In the proposed model, it is assumed that the fraction f remains constant irrespective of the axial

position z along the column. Accordingly, the product $(1 + f)P_{heat}$ is the total amount of heat lost by the mobile phase due to expansion cooling and to external heat exchanges. For the sake of simplicity, the radial temperature profile is assumed to be fully developed^{2,3} and the expressions of $\Delta_L T$ and $\Delta_R T$ become:

$$\Delta_L T = (1 + f)(1 + \langle \alpha T \rangle) \frac{\Delta P}{\langle C_p \rangle} \quad (5)$$

$$\Delta_R T = -f(1 + \langle \alpha T \rangle) \frac{\langle F_v \rangle \Delta P}{4\pi L \langle \lambda_{eff} \rangle} \quad (6)$$

The column and its surroundings eventually reach a steady state temperature regime for any selected flow rate. The radial heat flux, $\Phi > 0$, crossing any concentric cylinder of length L in the packed bed ($0 < r < r_c$), in the stainless steel column tube ($r_c < r < r_e$), in the region filled with air ($r_e < r < R_i$), and in the stainless steel housing tube ($R_i < r < R_e$) is then constant. It is written:

$$\Phi = -f(1 + \langle \alpha T \rangle) \langle F_v \rangle \Delta P > 0 \quad (7)$$

For the sake of illustration, the thermal problem was previously schematized in Figure 1 of reference¹⁹. For a given thermal environment in between the two coaxial stainless steel tubes (insulating polyethylene foam, air at atmospheric pressure, or air at a reduced pressure of 10^{-5} Torr), the solution to the problem consists in determining unambiguously the above-defined fraction f so that the temperature boundary condition far from the column (ambient lab temperature $T_\infty = 297$ K) is met. The exact expressions for the radial temperature profiles were previously reported in reference¹⁹ for each region of the system including the packed bed, the stainless steel column tube, the housing air, and the stainless steel vacuum tube.

3 Experimental

3.1 Chemicals

The mobile phase used was industrial carbon dioxide (99.8% pure) purchased from Airgas (Worcester, MA, USA). The *n*-heptane or acetonitrile solvents used as sample solvents were both HPLC grade from Fisher Scientific (Fair Lawn, NJ, USA). The sample mixture was a home-made mixture of seven *n*-alkylbenzenes prepared by dissolving benzene (C₀, 0.5 g/L), ethylbenzene (C₂, 0.7 g/L), butylbenzene (C₄, 1.1 g/L), hexylbenzene (C₆, 1.8 g/L), **octylbenzene** (C₈, 2.2 g/L), decylbenzene (C₁₀, 3.4 g/L), and dodecylbenzene (C₁₂, 4.5 g/L). Tetradecylbenzene (C₁₄, 2.0 g/L) and octadecylbenzene (C₁₈, 4.0 g/L) were prepared in *n*-heptane. Each *n*-alkylbenzene was purchased from Sigma-Aldrich (Suwanee, GA, USA) with a minimum purity of 99%.

3.2 Instrument and materials

The ACQUITY UPC² system (Waters, Milford, USA) was used to record the concentration profiles of seven *n*-alkylbenzenes. All the parts are those of the standard configuration of the ACQUITY UPC² system, except for the 8 μ L UV-Vis detection cell which was replaced with an external on-capillary (180 μ m i.d.) detection system as described in a previous report³¹. This enabled us to minimize post-column sample dispersion in the large detection cell volume. The system includes a binary pump with solvent selection valves and a two-valves injection system. A 1 μ L loop was used and the full loop injection mode was selected. The volume of the eluent mixer is 50 μ L. The ACQUITY UPC² system is controlled by the Empower software 3.0 (Waters, Milford, USA). An updated version of this software was used in order to measure accurately the second central time moments of the recorded concentration profiles.

The insulation pipe (Tubolit polyethylene, 25 mm wall thickness thick, 10 mm i.d., 1.83 m long) was purchased from Armacell LLC (Mebane, NC, USA).

The details about the materials required for the assembling of the home-made vacuum housing were already reported in¹⁹. The vacuum chamber (25 cm $\times$ 6 cm i.d.) may be pumped by a nominal 60 L/s turbomolecular pump (model TMH 071P, 90 000 rpm) from Pfeiffer Vacuum

(Asslar, Germany). The turbomolecular pump is used to generate pressures as low as 5×10^{-6} Torr in about an hour. No one vacuum gauge technology is optimal across the full range of air pressure investigated (5×10^{-6} Torr to 750 Torr). Therefore, pressure in the vacuum chamber was measured by a collection of gauges: three capacitance diaphragm gauges (models 600A-10T, 600AB-100T, and 600AB-1000T with full scale sensitivities of 10 Torr, 100 Torr and 1000 Torr respectively), a Pirani gauge (model APG-L, 10^{-4} to 5 Torr range) and an inverted magnetron gauge (AIM-C, less than 10^{-3} Torr). The gauges were controlled by an Active Gauge Controller. All gauges and the controller were obtained from Edwards High Vacuum (Crawley, UK).

The external surface area of the chromatographic column and most of the inner wall of the vacuum housing were covered with a thin and low emissivity aluminum tape. Aluminum-coated polyester tape was selected and purchased from 3M (Saint Paul, MN, USA). While 3M does not specify the emissivity, it is generally reported by users as about $\epsilon_{Al} = 0.04$. For the sake of comparison, the emissivity of machined and polished stainless steel 316 at 297 K is about $\epsilon_{SS}=0.28$.

3.3 Columns

A 3.0×150 mm column packed with $1.8 \mu\text{m}$ fully porous HSS-SB-C₁₈ particles from Waters (Milford, MA, USA) was used in this work.

3.4 Chromatographic experiments

A low-density SFC eluent (100% carbon dioxide) was selected in order to deliberately trigger thermal phenomena caused by the eluent decompression from 300 bar to 100 bar and by the temperature difference between the column wall and the ambient external surroundings. The external lab temperature was ambient around $T_{\infty}=297 \pm 1$ K. The inlet temperature of carbon dioxide was increased from $T_{inlet}=24$ °C to $T_{inlet}=107$ °C, and the active back pressure regulator (ABPR) was always set constant at 1500 psi for all chromatographic runs. The air pressure in the housing chamber was set at either atmospheric pressure (760 Torr) or at reduced pressure around 10^{-5} Torr (high vacuum).

In a series of experiments, the oven temperature was independently controlled from the mobile

219 phase temperature, which was set by a home-made mobile phase active preheater. The oven
220 temperature was increased step-wise from 30 to 40, 50, 60, 70, 80, 90, and to 100 °C. Similarly, the
221 inlet temperature of carbon dioxide was increased from 30 to 100 °C by steps of 10°C. Therefore,
222 a total of $8 \times 8 = 64$ thermal experiments were carried out. The changes in retention, peak shape,
223 and efficiency of the chromatographic column were recorded and investigated.

224 For all experiments, the flow rate of pure carbon dioxide was fixed at 2.1 mL/min (90% volume
225 fraction from pump head A) at 13 °C and at the observed system pressure which may depend on the
226 system configuration (mobile phase temperature, oven temperature, and housing chamber). Note
227 that 10% of the total flow rate comes from pump head B, e.g., 0.233 mL/min of pure 2-propanol was
228 delivered directly to the ABPR in order to continuously wash the post-column tubings and to avoid
229 any system over-pressure due to sample precipitation at low pressure and ambient temperature.
230 The reported retention time (t_R) and the **United States Pharmacopeia** (USP) efficiency (N_{obs})
231 were both directly taken from the standard processing method of the Empower software.

4 Results and Discussion

In the first part of this section, the onset of performance loss (analysis of the peak deformation of seven homologous compounds, from that of benzene to that of *n*-dodecylbenzenes) is determined experimentally when using low-density mobile phases under the standard configuration of the ACQUITY UPC² system (the oven temperature matches the mobile phase temperature). The inlet temperature of pure carbon dioxide and the oven temperature are equal and gradually increased from 30 to 100 °C. The flow rate (2.13 mL/min), the ABPR pressure (100 bar), and the thermal environment surrounding the column (oven still-air conditions) were all kept the same. In the second part, the independent control of the oven and mobile phase temperatures is utilized in order to limit the efficiency loss due to the thermal effects associated with the use of low-density carbon dioxide. In the third part, the potential benefits of either wrapping the column with an insulating material (2.5 cm thick polyethylene insulation sleeve) or applying a high vacuum (10⁻⁵ Torr, turbo-molecular pump) around the column walls are evaluated from the calculated reduction of the intensity of the radial temperature gradients. Finally, the experimental peak shape and USP efficiencies recorded for each insulation strategy are reported, compared, and discussed in line with the theoretical predictions.

4.1 Experimental emergence of peak distortion

Figure 1 show the chromatograms (three replicate injections in solid black, blue, and green lines) of seven *n*-alkylbenzenes recorded at eight different oven temperatures (30 °C, 40 °C, 50 °C, 60 °C, 70 °C, 80 °C, 90 °C, and 100 °C). The configuration of the ACQUITY UPC² system is standard, e.g., the oven temperature matches exactly the inlet mobile phase temperature and the column is placed horizontally in still-air inside the oven compartment.

First, it is remarkable that the peak shapes of all compounds remain excellent for any temperature smaller or equal to 60 °C. As expected, the retention time of each *n*-alkylbenzene is increasing with increasing the temperature. It is because the average density of carbon dioxide along the column is decreasing from about 0.9 g/cm³ at 30 °C to only 0.5 g/cm³ at 100 °C. As expected, due to thermal effects previously discussed, the peak shape is visibly deteriorating for the most

retained compounds when the temperature reaches and exceeds about 70 °C (average density of carbon dioxide around 0.7 g/cm³). All the recorded USP efficiencies of the 3.0 × 150 mm column packed with sub-2 μm particles are gathered for all compounds as a function of the temperature in Figure 2. The trend is very clear: the maximum performance of the SFC column (reduced plate height of 2.0, $N \simeq 35000$ -40000 theoretical plates) is reached for all compounds when the system temperature remains less than 50°C, e.g., when the CO₂ density remains large enough (> 0.75 g/cm³), even at the column outlet (100 bar). Accordingly, retention remains weak ($k' < 1$) and the steepness of the density gradients across the column diameter are not severe. They do not impact SFC performance significantly.

However, at temperatures higher than 60 °C, the retention of the analytes rapidly increases due to the significant diminution of carbon dioxide density. Additionally, the Joule-Thomson coefficient of the pure carbon dioxide (or its rate of cooling per unit of pressure drop along the column) is dramatically increasing and leads to undesirably high temperature differences between that of the cool column wall and that of the hot air inside the oven compartment. This results in a significant transverse heat exchange, radial temperature gradients, density gradients across the bed, and, therefore, in an unacceptable band deformation as shown in Figure 1 for $T=80$ °C, 90 °C, and 100 °C. **The explanation for such a peak split is as follows: the sample molecules traveling in the center of the column (cool) migrate at a faster speed than those progressing along the wall region of the column (hot). The recorded peak shapes allow to distinguish between these two groups of molecules while this pseudo peak split becomes even more dramatic as the retention factor of the *n*-alkylbenzene is getting larger.** The efficiencies reported in Figure 2 rapidly decrease to unacceptable values smaller than 1000 plates. It is noteworthy that the heavier the *n*-alkylbenzene compound is, the lower the temperature at which the column efficiency becomes less than half the maximum intrinsic efficiency. The effect is more noticeable for more retained components. Note that the efficiency measured for benzene never falls below 20 000 because benzene is never significantly retained, even at 100 °C ($k'=0.2$).

For the sake of interpretation of these observations, Figure 3 represents the evolution of the physical state of carbon dioxide in the pressure-temperature space (see the thick solid black lines)

as it decompresses from the column inlet (the inlet temperature increases from 30 to 100 °C) to the column outlet (the ABPR pressure was constant and set at 100 bar). The solid black lines were drawn from the outlet column temperature, which was measured with a surface thermocouple attached to the outlet capillary immediately after the outlet column endfitting, and from the inlet pressure provided by the recorded system pressure of the UPC² instrument. The solid blue lines represent the isopycnic (constant density in g/cm³) lines³². This graph returns the intensity of average density of carbon dioxide during its decompression along the column. The average density data are directly correlated to the retention of the seven *n*-alkylbenzenes. As the average density of carbon dioxide is decreasing, the Joule-Thompson coefficient is increasing and the thermal effects are becoming more significant.

To summarize, under standard column configuration where the column is placed in still-air (1 atm) inside the oven compartment and the oven temperature matches exactly the mobile phase temperature, the application of very low-density CO₂ mobile phases (T > 70 °C, 100 bar ABPR), which enable the experimenter to increase the retention and resolution of semi-volatile compounds, is inevitably accompanied by a certain drop in column performance. This originates from the finite amount of heat exchanged between the column wall and the surrounding air in the oven leading to the formation of steady state temperature and density gradients across the column i.d. The next sections aim at minimizing or eliminating the impact of such radial density heterogeneities on column performance.

4.2 Decoupling oven and inlet mobile phase temperatures

The column performance cannot be optimal with low-density mobile phases when the temperature of the oven compartment matches exactly the temperature of the mobile phase imposed at the column inlet. Indeed, the cooling of the mobile phase induces a diminution of the temperature of the stainless steel column wall and a negative temperature deviation from that of the surrounding air is eventually observed. This temperature difference is the main driving force for the generation of density gradients across the packed bed.

In this section, we propose to control the temperature of the oven compartment independently

from that of the mobile phase imposed at the column inlet. This can be done experimentally by using two independent active eluent preheaters: one is controlling the oven temperature while the second is independently fixing the actual temperature of the eluent entering the column. The main incentive here is to reverse the sign of the temperature difference between the column wall and air inside the oven at some distance along the 15 cm long stainless steel wall of the column. In the end, it is hoped that a compensation effect should be taking place regarding the relative migration velocity of the analytes at the wall and in the central region of the packed bed.

Accordingly, $8 \times 8 = 64$ temperature pairs for the air oven (30°C, 40°C, 50°C, 60°C, 70°C, 80°C, 90°C, and 100°C) and the inlet mobile phase (the same eight temperatures) were applied experimentally. Figure 4 is showing the resulting efficiency (heat map) for the seven injected *n*-alkylbenzenes (from benzene C0 to *n*-dodecylbenzene C12): in each of the seven graphs, the oven temperature is increasing from 30 to 100°C along the *x*-axis from right to left. The inlet temperature of carbon dioxide entering the column is increasing from 30 to 100°C along the *y*-axis from bottom to top. The efficient scale is given in the bottom right graph. The dotted black diagonal represents the conventional operating conditions of the ACQUITY UPC² instrument where oven temperature (T_{oven}) and mobile phase temperature (T_{inlet}) are exactly matching. Intrinsically, at best, such a 150 mm long column packed with 2 μm (accurately measured by scanning electron microscopy) fully porous HSS-SB-C₁₈ particles can provide a maximum efficiency of $150\,000\,\mu\text{m} / (2 \times 2\,\mu\text{m}) = 37\,500$ plates (dark red color). Interestingly, column efficiencies larger than 40 000 plates (brown color) have even been observed for most compounds when the difference $T_{inlet} - T_{oven}$ is positive and equal to about a few tens of degrees. This supports the anticipated result that the inlet temperature should always be larger than the oven temperature if one desires to reverse the sign of the temperature difference between that of the stainless steel column wall and that of air inside the oven after a certain distance from the column inlet. Along the first segment of the column length, the temperature is warmer in the column center than near the column wall (so, the retained analytes are eluted faster at the wall than in the bulk region of the packed bed) and *vice-versa* along the second segment. For that all, the observations in Figure 4 reveal that it remains extremely challenging to maintain the maximum column efficiency at the highest temperatures, especially for

the most retained compounds: the purple, blue, and green surface area are growing as the retention of the analyte gets larger. This compensation approach is only partly successful because it does not fully eliminate the nefarious radial heat flux across the bed diameter, source for the loss in column performance.

In conclusion, Figure 5 represents the column efficiency (heat map) as a function of the temperature difference $T_{inlet} - T_{oven}$ (x -axis) and retention factor k' (y -axis). The thick solid white line locates the visible optimum values of $T_{inlet} - T_{oven}$ maximizing column performance. It is clear that these optima are observed for positive temperature differences but that the maximum intrinsic efficiency ($\simeq 35000$ - 40000 plates) of the SFC column can not be achieved when the retention factor exceeds 1. $T_{inlet} - T_{oven}$ has to be set mostly in between 10 and 20 degrees in order to generate $N \simeq 40\,000$ for $k' < 1$. For larger k' values up to 10, the optimum column efficiency can no longer exceed 10 000 plates and the temperature of carbon dioxide should be about 30 degrees larger than the oven temperature. For instance, under conventional operating conditions at $T=80\text{ }^{\circ}\text{C}$, the retention factor of *n*-dodecylbenzene is about 4 and $N=1500$. The same retention factor is observed for $T_{oven}=60\text{ }^{\circ}\text{C}$ and $T_{inlet}=90\text{ }^{\circ}\text{C}$ and the improved efficiency is $N=9500$, a six time larger column performance. Figure 6 illustrates the advantage of being able to set independently the oven and inlet temperatures by comparing the chromatograms (three replicates) observed from one to the other system configurations.

In the next sections, alternative strategies are employed to reduce or even fully eliminate the source of the problem, e.g., the amount of heat exchanged between the column and the surrounding air.

4.3 Minimizing the heat exchanges between the column and the surrounding column environment

The first immediate approach to reduce heat transfer from the column wall to its surrounding environment is to wrap the column with an insulating material such as a thick (2.5 cm o.d.) Tubolit polyethylene foam (thermal conductivity 0.036 W/m-K). The incentive is to eliminate natural air convection while keeping heat conduction very small (the thermal conductivity of air is

0.026 W/m-K). A second and more effective solution will consist in applying a high vacuum (10^{-5} Torr) around the column wall so that heat transfer carried by conduction can also be eliminated. Additionally, heat radiation is minimized by wrapping the external surface area of the column with aluminum tape ¹⁹ (low emissivity coefficient $\epsilon=0.04$ *versus* $\epsilon=0.28$ for polished stainless steel 416). This approach has been recently and successfully applied in vHPLC when an excessive amount of viscous heat ($\simeq 15$ Watt/m) is produced inside the column at high pressure drops (13 000 psi) and high flow rates ($\simeq 1$ mL/min) along 2.1 and 3.0 mm i.d. columns. All the experimental details are given in the experimental section of reference ¹⁹.

Figure 7 shows the calculated radial temperature profiles (from the bed center at $r = 0$ to $r = \infty$ at ambient lab temperature) when applying these two different techniques (Tubolit polyethylene foam: solid green line; high vacuum: solid red line) designed to thermally insulate the chromatographic column from its surroundings. For the sake of reference, the same calculations were performed under standard conditions (air pressure 1 atm, solid blue line). In all thermal configurations, a low-density mobile phase (100% carbon dioxide) was sent to the column at 107 °C while the ABPR pressure was kept low at only 100 bar. The mathematical solution for the determination of the radial temperature profiles is given in the theory section based on an approximate model of heat transfer along and across the chromatographic column. Note that the external diameter of the column was 8 mm (3 mm i.d. and 15 cm long). Remarkably, the mere insulation of the column with the Tubolit polyethylene foam is not drastically efficient regarding the reduction of the temperature gradients across the packed bed: they decrease from 10.4 °C/cm to 6.5 °C/cm. This relative diminution of -37% is well correlated to the ratio of the thermal conductivities of air at atmospheric pressure (0.026 W/m-K) to that of Tubolit polyethylene (0.036 W/m-K). However, the impact of high vacuum (10^{-5} Torr) on the thermal insulation of the chromatographic column is much more efficient as previously reported experimentally in vHPLC ²⁰: the temperature gradients are reduced down to only 1.1 °C/cm (-90%) according to the calculations.

Experimental SFC data using pure carbon dioxide as the mobile phase are confirming the outcome of these calculations. The inlet temperature of carbon dioxide was set at 107 °C and the ABPR pressure fixed at 100 bar. The same HSS-SB-C₁₈ column was used at a flow rate

of 2.1 mL/min at 13°C and at the observed system pressure ($\simeq$ 300 bar). Figure 8 shows the chromatograms of the seven *n*-alkylbenzenes when only changing the thermal environment of the chromatographic column. Three different thermal environments were tested :

- 1) The bottom chromatogram (green color) shows the reference observations when the column is placed under standard still-air conditions (the ambient lab temperature is $T_{\infty}=297$ K, the air pressure is 1 atm). Similarly to the chromatogram shown in Figure 1 at 100 °C (the inlet and oven temperatures were the same), it is noteworthy that the peak shape and the USP efficiencies of the most retained compounds are visibly poor with respect to those expected for such a high efficient column packed with sub-2 μ m particles. Note also that the temperature of the eluent measured at the column outlet is only 44 °C, a value much smaller than 57 °C had the column been operated under strict adiabatic conditions. Therefore, in addition to the expansion cooling of the eluent during its decompression from 4360 psi to 1500 psi, the measurement of the outlet eluent temperature indirectly confirms that carbon dioxide further cools down due to the heat released to the external environment by natural air convection and surface radiation.

- 2) The middle chromatogram (blue color) was recorded in the presence of the thick (2.5 cm) polyethylene insulation sleeve. Natural convection is then eliminated. Most remarkably, such a thermal insulation of the column body is still insufficient to provide the user with acceptable peak shapes and column efficiencies. Indeed, the temperature of carbon dioxide at the column outlet (48 °C) is still smaller than 57 °C: this means that the amount of heat exchanged by pure conduction through the polyethylene foam remains significant. This agrees well with the previous calculations regarding the prediction of a moderate reduction of the temperature gradient steepness across the packed bed when wrapping the column with this insulation material.

- 3) The top chromatogram (black color) was obtained after applying a high-vacuum (10^{-5} Torr) in the housing chamber surrounding the column. It is remarkable that, under such

conditions, the peak shapes of all compounds (even the most retained ones) return to quasi-symmetrical and the USP efficiencies reach their maximum. This observation is fully consistent with the elimination of air conduction^{19,20} and the predicted reduction of the temperature gradient steepness by a factor ten. Note that the eluent temperature at the column outlet (55 °C) is now very close to 57 °C, showing that the column is well placed in a quasi-adiabatic thermal environment.

Figure 9 shows the same chromatograms as those in Figure 8, except the injection of heavier compounds (*n*-tetradecylbenzene and *n*-hexadecylbenzene are dissolved in *n*-heptane). **This change in sample solvent has a consequence on the elution times of the *n*-alkanes. In fact, the retention times of C₁₄ dissolved in *n*-heptane are even smaller than those of C₁₂ dissolved in acetonitrile (see Figure 9). It is because acetonitrile is significantly retained onto the column while *n*-heptane is not²⁷.** They confirm the significant improvement in the peak shapes of these retained analytes when applying a high air vacuum at the column wall. The observed efficiencies are measured around 15 000 and 10 000 for retention factors as large as 10 and 25. They were initially smaller than 1500 even for smaller retention factors of about 4 and 9. **Note, however, that the signal-to-noise ratio is visibly smaller in the vacuum trial than in the other two trials. In fact, in order to avoid thermodynamic column overload (the peak shape can be deformed due to an anti-langmuirian adsorption behavior) the concentration of the injected sample in *n*-heptane was reduced by a factor 3. This explains the relatively lower signal-to-noise ratio observed in the vacuum trial.**

Finally, Figure 10 compares the evolution of the physical state of carbon dioxide as it decompresses along the column for each above-mentioned thermal environment (see the solid green, blue, and black lines). It quantifies for a selected SFC thermal environment how much the system deviates from strict adiabatic conditions: in theory, if the column is placed under adiabatic conditions, then, the solid lines should run parallel to the solid red lines (isenthalpic transformation) represented in this graph. This is the case when a high air vacuum is applied around the column. This is clearly not the case for the other two thermal environments of the column because the experimental solid lines are crossing the isenthalpic lines: the larger the deviation is, the larger the amount of

452 heat exchanged between the column and the external environment is.

5 Conclusion

In this work, it has been shown that it is possible to maximize experimentally SFC column performance when low-density mobile phases (such as pure carbon dioxide at elevated temperatures combined with low ABPR pressures) are used to increase the retention of semi-volatile compounds. Under such experimental conditions, the usual observed loss in column performance originates from the large Joule-Thompson coefficient of these mobile phases, the large amount of heat exchanged between the column wall and its surrounding thermal environment, and from the formation of undesirable density gradients across the column diameter.

Two experimental set-ups happen to be partly and fully successful in order to improve the SFC column performance: 1) the independent adjustment of the oven temperature and of the mobile phase temperature enable the user to compensate for the radial density gradient across the column i.d. as the analyte is progressing along the column. This compensation effect refocuses the chromatographic band to some extent and increases the observed column efficiency by a factor 5 for a fixed retention factor close to 4. 2) the complete thermal insulation of the chromatographic column by application of a high vacuum (10^{-5} Torr) drastically improve the SFC column performance by a factor 30 for an even larger retention factor of about 10. It is because the problem is tackled at its source after fully eliminating the amount of heat lost at the column wall to the external environment. It was also observed that the mere insulation of the chromatographic column with the Tubolit polyethylene foam did not solve the efficiency problem because heat transfer by conduction through the insulating material remains too large.

In conclusion, the advantage of placing the chromatographic column under adiabatic conditions has been experimentally extended from LC (where the heat is released by viscous heating) to SFC (the heat is released by eluent expansion). The main application areas are the possibility of analyzing simultaneously by gradient SFC mixtures of semi-volatiles and heavy compounds (petroleum products, gasoline, terpenes in oils, paraffin wax,...). Very low-density carbon dioxide (elevated temperature > 100 °C combined with low ABPR pressure < 100 bar) can be used at the beginning of the SFC gradient to retain and separate efficiently the most volatile compounds while the high-molecular-weight analytes are resolved by continuously increasing the amount of organic

481 solvent (methanol, ethanol, ...) and/or the column back pressure.

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Figure captions

- 1 Experimental chromatograms (three replicate injections: black, blue, and green solid lines) of seven *n*-alkylbenzenes (from benzene C₀ to *n*-dodecylbenzene C₁₂) dissolved in acetonitrile for eight different oven temperatures (30°C, 40°C, 50°C, 60°C, 70°C, 80°C, 90°C, and 100°C). Standard configuration of the ACQUITY UPC² system where the oven temperature matches the inlet temperature of the mobile phase. Column: 3.0 mm × 150 mm 1.8 μm HSS-SB-C₁₈; mobile phase: 100% carbon dioxide; flow rate: 2.1 mL/min at 13°C and at the observed inlet column pressure that decreases from 5101 psi at 30°C to 4187 psi at 100 °C; detection wavelength: 243 nm, sampling rate 20 Hz. The column is placed in the oven compartment under still-air conditions. Note the progression of peak distortion as the temperature is increasing. 33
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577		mm column for eight different temperatures as indicated on the top of each one	
578		of the thick solid lines. Same experimental conditions as in Figure 1. Note that	
579		the average density of carbon dioxide along the column changes significantly from	
580		high density (small retention factor, high column performance) to low density (high	
581		retention factor, low column performance) carbon dioxide. The diminution of the	
582		column performance at high temperatures is related to thermal effects (temperature	
583		difference between the cool column wall and the hot air in the oven) and the subse-	
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590		was tested: the oven temperature is set independently from the inlet temperature of	
591		the mobile phase. Eight different temperatures (from 30°C to 100°C by step of 10°C)	
592		were applied for the column oven (<i>x</i> -axis, right-to-left) and for the mobile phase at	
593		the column inlet (<i>y</i> -axis, bottom-to-top). The dotted black diagonal represents the	
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598		alkylbenzenes are condensed in a single representation: the <i>y</i> - and <i>x</i> -axis account	
599		for the retention factor (<i>k'</i>) and the difference between the inlet and oven temper-	
600		atures (<i>T</i> _{inlet} - <i>T</i> _{oven}), respectively. This heat map reveals that the optimum value	
601		of <i>T</i> _{inlet} - <i>T</i> _{oven} (thick solid white line) increases from about +10 to +30 °C as the	
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604		on column efficiency at constant retention factor. (Top graph) Standard configu-	
605		ration of the ACQUITY UPC ² system: $T_{oven} = T_{inlet} = 80\text{ }^{\circ}\text{C}$. The efficiency of	
606		n -dodecylbenzene is 1500. (Bottom graph) Modified configuration of the ACQUITY	
607		UPC ² system where the oven temperature and the inlet mobile phase temperature	
608		can be set independently from each other: $T_{oven} = 60\text{ }^{\circ}\text{C}$ and $T_{inlet} = 90\text{ }^{\circ}\text{C}$. The	
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613		m) at the column outlet, the vacuum housing chamber (from $r=0.004$ to 0.030 m), the	
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615		environments of the $3.0\text{ mm} \times 150\text{ mm}$ $1.8\text{ }\mu\text{m}$ HSS-SB-C ₁₈ column, and across	
616		ambient lab air ($r > 0.084\text{ m}$). The details of the calculations are summarized in the	
617		theory section and in reference ¹⁹ . In blue color, the housing chamber is filled with	
618		air at atmospheric pressure (heat exchanges are carried by natural air convection).	
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620		foam (natural air convection is eliminated). In red color, the housing chamber is	
621		filled with air at a reduced pressure (10^{-5} Torr). In this last column configuration,	
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629		red and orange colors on the top of the graph indicate that the eluent	
630		temperature is decreasing from the inlet to the outlet. Note that the	
631		column temperature is always higher than room temperature. (Bottom	
632		graph, green color) The column is placed under standard still-air conditions. The	
633		heat exchanged is transported by natural air convection. (Middle graph, blue color)	
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646		under standard still-air conditions. In blue, the column is wrapped with a 2.5 cm	
647		thick polyethylene foam. In black, a high vacuum (10 ⁻⁵ Torr) is applied around the	
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650		lines.	42

Figure 1: Experimental chromatograms (three replicate injections: black, blue, and green solid lines) of seven *n*-alkylbenzenes (from benzene C0 to *n*-dodecylbenzene C12) dissolved in acetonitrile for eight different oven temperatures (30°C, 40°C, 50°C, 60°C, 70°C, 80°C, 90°C, and 100°C). Standard configuration of the ACQUITY UPC² system where the oven temperature matches the inlet temperature of the mobile phase. Column: 3.0 mm × 150 mm 1.8 μm HSS-SB-C₁₈; mobile phase: 100% carbon dioxide; flow rate: 2.1 mL/min at 13°C and at the observed inlet column pressure that decreases from 5101 psi at 30°C to 4187 psi at 100 °C; detection wavelength: 243 nm, sampling rate 20 Hz. The column is placed in the oven compartment under still-air conditions. Note the progression of peak distortion as the temperature is increasing.

Figure 2: Plot of the USP column efficiency (N) of the seven n -alkylbenzenes (from benzene C_0 to n -dodecylbenzene C_{12}) as a function of the oven temperature (from 30 °C to 100 °C). Same experimental conditions as those given in Figure 1. Note the diminution of the column performance at high temperatures for low-density carbon dioxide mobile phase and the most retained analytes.

Figure 3: Evolution of the physical state of carbon dioxide (thick solid black lines) in a pressure-temperature representation during its actual decompression along the 3.0 mm $\times$ 150 mm column for eight different temperatures as indicated on the top of each one of the thick solid lines. Same experimental conditions as in Figure 1. Note that the average density of carbon dioxide along the column changes significantly from high density (small retention factor, high column performance) to low density (high retention factor, low column performance) carbon dioxide. The diminution of the column performance at high temperatures is related to thermal effects (temperature difference between the cool column wall and the hot air in the oven) and the subsequent formation of density gradients across the column diameter. The thin solid blue lines represent isopycnic (or constant density in g/cm³) lines. The decompression of carbon dioxide is strictly adiabatic along an isenthalpic line.

Figure 4: Heat maps associated with the USP efficiency of the 3.0 mm $\times$ 150 mm 1.8 μ m HSS-SB-C₁₈ column measured for seven *n*-alkylbenzenes (from benzene C0 to *n*-dodecylbenzene C12). A prototype configuration of the ACQUITY UPC² system was tested: the oven temperature is set independently from the inlet temperature of the mobile phase. Eight different temperatures (from 30°C to 100°C by step of 10°C) were applied for the column oven (*x*-axis, right-to-left) and for the mobile phase at the column inlet (*y*-axis, bottom-to-top). The dotted black diagonal represents the conventional configuration of the ACQUITY UPC² system where oven temperature matches the inlet eluent temperature. Same other experimental conditions as in Figure 1. Note that most efficiency maxima are located above the diagonal.

Figure 5: Same as in Figure 4, except all seven heat maps for the USP efficiencies of all n -alkylbenzenes are condensed in a single representation: the y - and x -axis **account** for the retention factor (k') and the difference between the inlet and oven temperatures ($T_{inlet} - T_{oven}$), respectively. This heat map reveals that the optimum value of $T_{inlet} - T_{oven}$ (thick solid white line) increases from about +10 to +30 °C as the retention factor of the analyte is increasing from 0.3 to about 10.

Figure 6: Impact of adjusting independently oven temperature and mobile phase temperature on column efficiency at constant retention factor. (Top graph) Standard configuration of the ACQUITY UPC² system: $T_{oven} = T_{inlet} = 80\text{ }^{\circ}\text{C}$. The efficiency of *n*-dodecylbenzene is 1500. (Bottom graph) Modified configuration of the ACQUITY UPC² system where the oven temperature and the inlet mobile phase temperature can be set independently from each other: $T_{oven} = 60\text{ }^{\circ}\text{C}$ and $T_{inlet} = 90\text{ }^{\circ}\text{C}$. The USP efficiency of the most retained compound, *n*-dodecylbenzene, increases from 1500 to 9500.

Figure 7: Calculated radial temperature profiles ($T(r)$, solid colored lines) across the packed bed (from $r=0$ to 0.0015 m), the stainless steel column tube (from $r=0.0015$ to 0.004 m) at the column outlet, the vacuum housing chamber (from $r=0.004$ to 0.030 m), the stainless steel housing tube (from $r=0.030$ to 0.0384 m) for three different thermal environments of the $3.0\text{ mm} \times 150\text{ mm}$ $1.8\text{ }\mu\text{m}$ HSS-SB- C_{18} column, and across ambient lab air ($r > 0.084$ m). The details of the calculations are summarized in the theory section and in reference ¹⁹. In blue color, the housing chamber is filled with air at atmospheric pressure (heat exchanges are carried by natural air convection). In green color, the housing chamber is filled with a Tubolit polyethylene insulation foam (natural air convection is eliminated). In red color, the housing chamber is filled with air at a reduced pressure (10^{-5} Torr). In this last column configuration, heat transported by conduction is eliminated and heat exchanges are only supported by residual surface (aluminum tape) radiation.

Figure 8: Effect of the thermal insulation of a SFC column on its resolution performance. Separation of seven *n*-alkylbenzenes from C0 (benzene) to C12 (*n*-dodecylbenzene dissolved in acetonitrile. Column: 3.0 mm $\times$ 150 mm packed with 1.8 μ m HSS-SB-C₁₈. Inlet temperature of carbon dioxide: 107 °C. ABPR pressure: 100 bar. Ambient lab temperature and pressure: 24 °C, 1 atm. 1 μ L injection volume. **The red and orange colors on the top of the graph indicate that the eluent temperature is decreasing from the inlet to the outlet. Note that the column temperature is always higher than room temperature.** (Bottom graph, green color) The column is placed under standard still-air conditions. The heat exchanged is transported by natural air convection. (Middle graph, blue color) The column is wrapped with a 2.5 cm thick insulating material, Tubolit polyethylene foam. Natural air convection is eliminated. (Top graph, black color) A high vacuum (10^{-5} Torr) is applied around the column. Heat transport by conduction is eliminated and heat is only carried by residual surface (aluminum tape) radiation.

Figure 9: Same as in Figure 8, except the separation of two heavier C14 and C18 *n*-alkylbenzenes dissolved in *n*-heptane. Note, despite a larger retention, the drastic improvement in the peak shape and symmetry of both compounds when the column is thermally insulated under high vacuum (10^{-5} Torr). Radial density gradients across the SFC column are virtually eliminated.

Figure 10: Evolution of the molar enthalpy of carbon dioxide (thick solid colored lines) in the pressure/temperature representation during its decompression along the HSS-SB-C₁₈ column for three different thermal environments. In green, the column is placed under standard still-air conditions. In blue, the column is wrapped with a 2.5 cm thick polyethylene foam. In black, a high vacuum (10⁻⁵ Torr) is applied around the column. The thin solid red lines represent the isenthalpic (or constant enthalpy in J/g) lines. The decompression of carbon dioxide is strictly adiabatic along isenthalpic lines.