



Use of isopycnic plots in designing operations of supercritical fluid chromatography: II. The isopycnic plots and the selection of the operating pressure–temperature zone in supercritical fluid chromatography

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ABSTRACT

In SFC, the key chromatographic parameters, the retention factors and the column efficiency, strongly depend on the density of the mobile phase. This indicates that the isodensity or isopycnic plots, drawn on the pressure–temperature plane, can provide an effective tool to help analyzing how the chromatograms obtained in SFC evolve, when the experimental conditions, the inlet and outlet pressures and the column temperature, are changed. In a companion paper, we analyzed the role of density in controlling the physical properties of the mobile phase, which in turn controls solute retentions and column efficiencies. In this report, we analyze the operating conditions in SFC with reference to the isopycnic plots of carbon dioxide. This analysis clarifies the differences and similarities between the operating conditions selected in the subcritical zone and those located in the supercritical zone. It also sets out an operational map illustrating how retention factors vary with respect to the operating temperatures and pressures. This study is focused on the use of pure carbon dioxide as the mobile phase, but the same method of investigation is also applicable when the mobile phase contains a modifier.

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1. Introduction

Within the ranges of temperature and pressure that are normally applied in separations made by SFC, the physical properties of the mobile phase can be modulated to a considerable extent. This tunability of CO₂ as a solvent provides the opportunity of using the same eluent but adjusting its physico-chemical properties in a wide range of values, through the proper selection of the operating temperatures and pressures. This great facility, however, is also the source of a major challenge for analysts and process designers who need to determine the most suitable operating conditions.

Historically, interest in the design of analytical or preparative separations by SFC has mostly been focused on conducting chromatography under supercritical conditions. Considerable effort was invested in trying to work in the supercritical zone of CO₂, which is shown by the shaded area on the pressure–temperature plane in Fig. 1. The primary purpose of these efforts was to tap the properties of supercritical CO₂, i.e., its low viscosity and the high diffusivity of solutes dissolved in it, in order to achieve chromatographic separations faster than those achieved in HPLC.

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Although the supercritical zone is defined by the isothermal and the isobaric lines passing through the critical point, it was realized later that there are no magical properties associated with this zone, as it was believed by some in the 1980s. Actually, the critical point is essentially a singular point, where the differential of many important properties or functions are nought or infinite but neither the critical isothermal line nor the critical isobaric line separate the pressure–temperature plane in zones of abruptly different properties. Crossing either of these two lines does not result into any phase change and there are no abrupt difference between the properties of the high density gas, the liquid, and the supercritical fluid. In contrast there is a smooth, continuous transition across the whole pressure–temperature domain [1–3]. Separating the grey quadrant in Fig. 1 as a special operating zone, as was suggested in the past, is arbitrary. From this observation, Martire developed a unified theory of chromatography that applies without any distinction between the state of the fluid, whether it is named and considered as a gas, a liquid or a supercritical fluid [1,4]. Later, Chester [5–7] expanded this observation, noting that if either the temperature or the pressure is kept above its critical value during the experiments, one can implement a seamless variation of the mobile phase properties without any discontinuity between gas, liquid or supercritical chromatography.

Table 1
Properties of supercritical CO₂ [2].

	Temperature (°C)	Pressure (bar)	Density ^a (g/mL)	Diffusivity (cm ² /s)	Viscosity (cP)
Low density	100	80	0.15	10 ⁻³	0.02
High density	35	200	0.8	10 ⁻⁴	0.1

^a The critical density of CO₂ is 0.45.

Per se, however, this understanding does not help much in estimating the operational possibilities in the subcritical and supercritical zones nor in predicting the possible differences in the experimental results that could be expected when selecting either zone for the experimental conditions. In fact, the capabilities of supercritical CO₂ as a chromatographic mobile phase remain unspecific and operating in the critical range of supercritical conditions is often times overly rated. Frequent references to the supercritical properties as being *intermediate* between gas and liquid properties and the notion that the supercritical fluid combines the advantages of both those of a liquid, like a high solvating power, and a gas, like a high fluidity, mainly contributed to profound misunderstandings. Schoenmakers [2] observed the incompleteness of this notion, preferring to divide the supercritical zone into a “high-density” and a “low-density” parts, as expressed by the data set shown in Table 1. He noted that it is incorrect to define and refer to a hypothetical supercritical fluid, attributing the best properties (the high density in the first row and the high fluidity in the second row of Table 1) of the two different sets to one single fluid [2].

Experimental studies conducted over various pressure and temperature ranges in the supercritical zone of CO₂, however, helped in developing some thumb-rules regarding the choice of operating conditions. For example, working with low-density CO₂ had drawn interest due to the low viscosity and the high diffusivity in that zone, because it was expected that this combination of properties would lead to fast, highly efficient separations. However, the uncertainty of working there was soon pointed out by Berger [8], who noted that the efficiency of columns operated in the part of the supercritical zone close to the critical region, at temperatures lower than 40 °C and pressures lower than 80 bar tends to be mediocre. He suggested, based on the experimental observations, that poor efficiencies would be observed in an approximately triangular zone of the supercritical region shown in Fig. 2. At temperatures 10–20 °C higher or under pressures 10–20 bar larger than these thresholds, however, far better results were to be observed. Poe [9] noted a sig-

nificant loss in the efficiency of packed columns operated at large pressure drops, when the inlet density was below 0.7 g/mL and the temperature below 100 °C. Rajendran et al. [10] reported that if the outlet pressure of an SFC packed column is decreased to 130 bar at temperatures between 328 and 338 K, the efficiency decreases abnormally and even becomes practically negligible around 338 K. All these observations suggest that there might be great potential disadvantages in working under experimental conditions too close to the critical region. Realization of this situation motivated analysts and process designers to choose operating pressures far above the critical pressure to avoid any unwanted outcome.

The addition of organic modifiers to CO₂ to increase the solubility of polar solutes gives another dimension to the selection process of suitable operating conditions. The critical properties of mixtures are significantly different from those of the pure main component [11,6]. So, it is clear that maintaining operating temperatures and pressures as guided by the CO₂ phase diagram alone does not ensure supercriticality of the mobile phase when modifiers are added. In other words, if the operating temperature and pressure are within the supercritical region of CO₂ but the mobile phase contains an organic modifier, the system performs most likely in the subcritical state of the mixture, because the critical points of organic solvents are significantly higher than the one of CO₂. This realization made the criterion of operating columns at temperatures above CO₂ critical temperature largely irrelevant. Several other advantages of working below CO₂ critical temperature were also reported, e.g. (a) the miscibility of CO₂ and organic modifiers is better under subcritical conditions because phase separation inside the column between modifier rich and modifier poor phases is more easily avoided [12] and (b) the compressibility of the mobile phase is much lower, so the decrease of the eluent den-

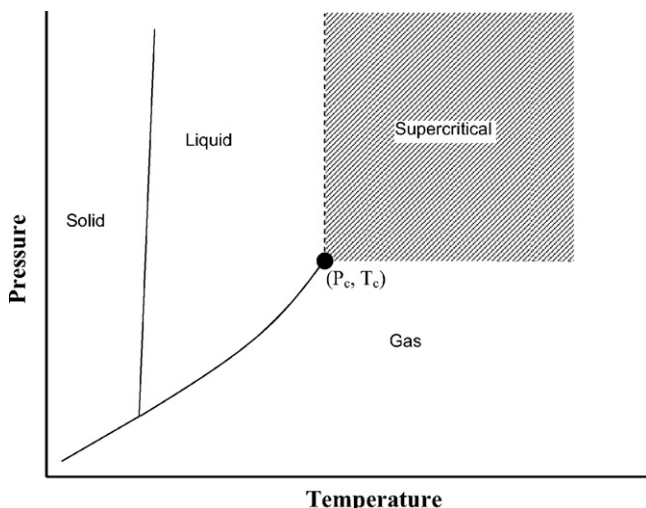


Fig. 1. Schematic diagram of supercritical zone on a pressure–temperature plane.

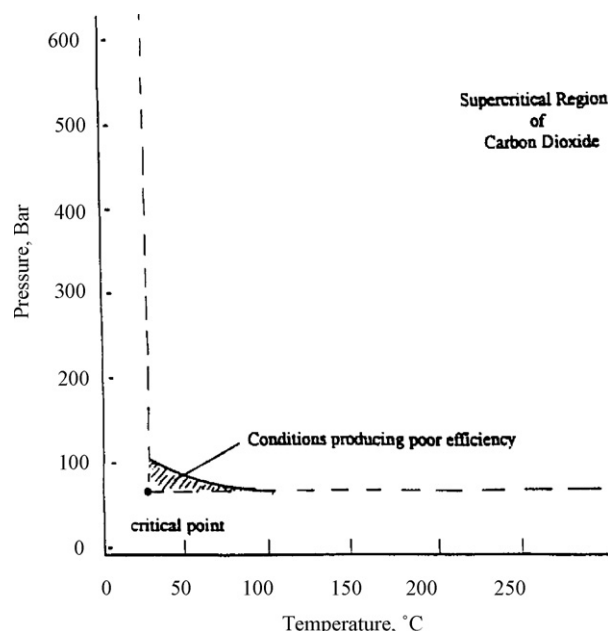


Fig. 2. Approximate region of poor efficiency as predicted by Berger [8].

sity taking place along the column is smaller, even when operating with a high pressure drop, which, in turn, ensures lesser variations of the local column efficiency [12], leading to more robust and stable separations. Eventually, such insights nudged the preferred operating zone of SFC toward temperatures below the CO₂ critical temperature, leading to the use of denominations such as subcritical, near-critical, or enhanced fluidity chromatography.

A recent review [13] illustrates that, apart from this preference of working under pressures much higher than the critical pressure and at subcritical temperatures, the selection of the experimental conditions in SFC, i.e., the choice of the operating pressures and temperatures, are generally not based on any specific understanding of the possible repercussions of working in that particular pressure–temperature region. As a consequence, it still remains uncertain how far from the critical point, and in which direction, it is possible to successfully operate SFC columns and to achieve rewarding separations of mixtures. With no clear understanding on this issue, no systematic procedure could be designed for selecting experimental conditions reasonably close to those providing optimum industrial separations.

The main objective of our current work is to develop a better insight into the consequences of working in different operating regions in SFC. The companion paper [14] shows that, although SFC behavior is largely controlled by the viscosity of the mobile phase and the diffusivity and the solubility of the solute molecules, these properties are actually controlled by the density and to some extent by the temperature. Taking a cue from this observation, this work attempts to show that it is easier to understand the consequences of adjusting the operating conditions by following the isopycnic lines plotted on the pressure–temperature plane, rather than by following the isotherms and the isobars themselves.

The analysis presented in this paper is based on the use of pure CO₂ as the mobile phase. Because CO₂ is the pivotal component of all the mobile phases used in SFC, the inferences drawn from this study are largely applicable to general SFC operations as well. Many inferences drawn in this study are qualitative in nature, but they offer valuable insights into the operations of the SFC process, including those cases in which the mobile phase contains significant concentrations of a modifier.

2. Application of the isopycnic plots

Separations performed in SFC depend essentially on the retention factors obtained for the sample components and on the column efficiency. The important role of the mobile phase density in determining these separation characteristics was highlighted by several researchers. While this role of density is certainly important, we underline in the companion paper [14] that, even at constant density, the temperature plays an important role in determining these parameters because SFC operations fundamentally depend on physical properties like the viscosity of the mobile phase and the diffusivity and solubility of the solutes. While the viscosity and the molecular diffusivities are strongly controlled by density, solubility is controlled by both density and temperature. Because solubility directly controls the retention factors and indirectly the column efficiency, both the density and the temperature play important roles in SFC separations [14]. This explains why chromatographic parameters can be expressed as simple functions of the mobile phase density and the column temperature, rather than as functions of the pressure and the temperature.

Guided by this observation, it was reasoned [14] that chromatographic separations can be investigated more clearly by considering the isopycnic lines plotted on the pressure–temperature plane rather than the isotherms and the isobars themselves. To evaluate this idea, we introduce the isopycnic plot (Fig. 3), a set of isopyc-

nic lines on the pressure–temperature plane of CO₂. These lines are drawn for densities increasing from 0.1 to 1.05 g/mL at an interval of 0.05 g/mL. They were generated from the REFPROP program [15], which uses the Span and Wagner [16] equation of state to calculate the density values. The uncertainty in the estimates of the density values varies from 0.03 to 0.05% at pressures up to 300 bar and temperatures up to 523 K [15]. So, these values can be considered as sufficiently accurate for most practical purposes in SFC. The experimental conditions of a separation in SFC are represented in the isopycnic plot by the operating point, which has the temperature and pressure as the coordinates.

Based on the proximity of the isopycnic lines and on the position of the critical isotherm, the part of this plot that is relevant to SFC separations can be divided into three zones, as indicated in Fig. 3. The critical point is marked by a solid circle. Zone A in Fig. 3 consists in the quadrant which is often referred to as the subcritical SFC zone, in which CO₂ is under a pressure higher than the critical pressure but at a temperature lower than the critical temperature. The quadrant combining zones B and C is the supercritical zone of CO₂. It is divided by the $\rho = 0.75$ g/mL isopycnic line. The main difference between zones B and C is in the average distances between the isopycnic lines. In zone B these lines are sparsely placed, indicating a zone of low compressibility. In contrast, the isopycnic lines in zone C are densely located, particularly in the vicinity of the critical point, indicating high compressibility.

The main usefulness of the isopycnic plots is to assist in understanding the consequences of the selection of the experimental conditions under which a SFC separation will be attempted. These conditions include generally the inlet and the outlet temperatures and pressures of the column and inform on the variations of the density and the temperature along the column. Understanding how these conditions affect a separation could allow improvements of this separation by indicating how should these conditions be altered in order to keep constant, e.g., the mobile phase density distribution along the column, or at least to minimize the changes in this density. From the orientations and the positions of the isopycnic lines, the following general observations can be made:

1. The relative position of an operating point with respect to the critical isotherm is irrelevant from the SFC operational point of view. Since the density and the temperature are the two prime controlling parameters of SFC operations [14], there is no fundamental difference between working at a subcritical or a supercritical temperature, because the isopycnic lines cut across the critical isotherm, creating similar operating zones that belong to both sub- and supercritical fluid chromatography. So, any distinction between subcritical or supercritical conditions, often made in the literature, is artificial.
2. The positions and the orientations of the isopycnic lines not only illustrate the continuity of the property variation across the critical isobar and the critical isotherm, which was already known for a long time, they also clarify the nature of this variation, which is markedly different along the pathways cutting across the critical isotherm and those cutting across the critical isobar. The isopycnic lines show that although this variation is continuous, it is not uniform; and it divides the pressure–temperature plane into zones within which the compressibility varies widely.
3. The isopycnic plots suggest that selecting operating conditions near the critical point has no obvious disadvantages, provided that the separation is not carried out in the high compressibility zone, i.e. zone C, which begins approximately at densities below $\rho = 0.75$ g/mL and at temperatures above the critical temperature. This means that an SFC separation should be more conveniently carried out right at the critical temperature rather

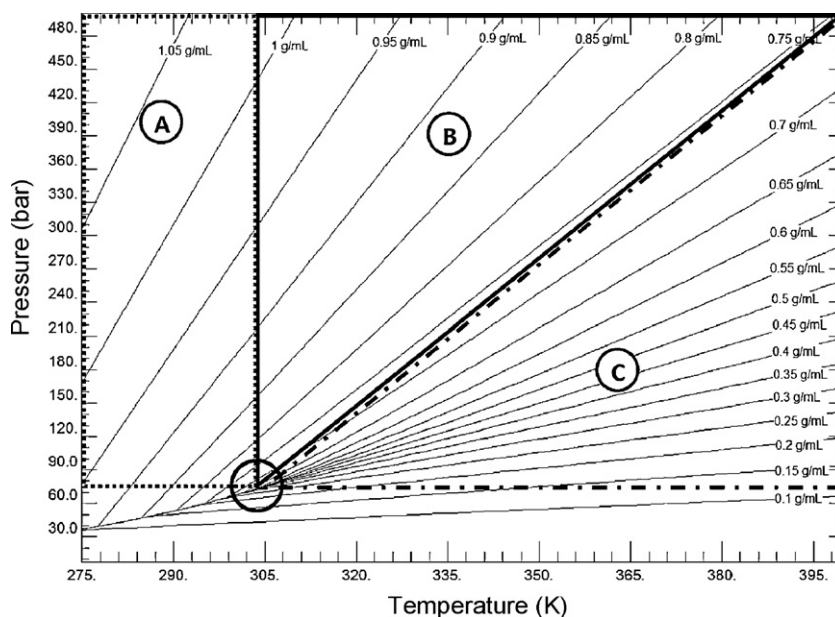


Fig. 3. Isopycnic lines ranging from 0.1 g/mL to 1.05 g/mL are plotted at an interval of 0.05 g/mL, on the pressure–temperature diagram of CO₂. The diagram above the critical isobar has been divided into three different zones. Zone A represents the sub-critical CO₂ region where the pressure is higher than the critical pressure but the temperature lower than the critical temperature. The supercritical region of CO₂ has been divided into zones B and C based on the relative proximity of the isopycnic lines.

than at some supercritical temperatures, if it has to be done at operating pressures close to the critical pressure.

It should be emphasized here that the observations made above regarding the consequences of selecting the operating point of a separation could not be developed without considering the isopycnic lines on the pressure–temperature plane. In the following subsections, we discuss the advantages and drawbacks of selecting operating points in the three different zones, A, B, and C described above.

2.1. Operations in zone A

In zone A, which covers the possible subcritical operating points in SFC, the density does not vary significantly even for a large pressure change, especially above 150 bar, where most of the industrial operations are conducted. The isopycnic lines passing through this zone at pressures in excess of 150 bar correspond to densities higher than 0.85 g/mL. The increase in density due to compression is less than 0.06%/bar at 273 K and less than 0.8% at 300 K. Although the viscosity of CO₂ increases by nearly 50% when the density increases from 0.85 to 1.0 g/mL (ref. to [14]), i.e. in this entire zone A, any viscosity change in this zone is linearly correlated to the density variation, making simpler the modeling of SFC operations in this zone. Even below 150 bar, the situation is not much different, although compressibility increases at the region close to the critical point.

The most prominent advantage of operating in this zone arguably is the low compressibility of subcritical CO₂. Pressure drops, even as high as 100 bar along the column, result in a small change in the density, hence in the other properties of CO₂ under isothermal or near isothermal conditions. This suggests that the challenges in SFC operations in the subcritical zone are not very different from those encountered in HPLC operations. The most prominent advantage of SFC over HPLC operation, however, is the lower viscosity of CO₂. Although CO₂ viscosity is larger in zone A (ca. 0.08–0.12 cP) than in either supercritical zone B or C, it is still about 5–20 times lower than that of the solvents frequently used in HPLC, e.g. water (1.0 cP), methanol (0.59 cP), or even acetonitrile

(0.38 cP). Even when organic modifiers are used, the viscosity of the mixed mobile phase is significantly lower than that of HPLC mobile phases. In other words, with many of the instrumental issues solved and instruments working reasonably well, most HPLC practitioners can now use SFC much like they do HPLC while enjoying the benefits brought by a mobile phase based on CO₂, which can be used at much higher flow rates, providing faster analyses or product separations [17,18]. Understandably, this incarnation of SFC will probably make significant inroads into the territory of HPLC for prominent applications. The difference between the non-aqueous CO₂ based mobile phase of SFC and the mainly aqueous mobile phases of HPLC is due to the different interactions between the solutes, the mobile and the stationary phases, which often leads to SFC and HPLC having orthogonal separation powers, which is another potential advantage [19].

2.2. Operations in zone B

Fig. 3 shows clearly that there are actually no significant differences between the isopycnic lines in zones A and B and particularly not in their slope and relative proximity. These lines are continuous straight lines crossing between these two zones without bending. As pointed out by Chester [6], there is no fundamental difference in the resultant behavior between operating in the subcritical or in the high-pressure supercritical regions. The isopycnic lines gradually fan out from the liquid state in the subcritical zone A to the supercritical state zone B, without exhibiting any discontinuity. The fluid compressibility evolves progressively and the boundary between these two regions may seem artificial. In fact, the compressibility is higher at the low-pressure part of the subcritical CO₂ region (zone A), near the critical temperature and at pressures lower than 150 bar, than at the high-pressure part of the supercritical region (zone B), near the critical temperature but at pressures above 300 bar (Fig. 3). So, the notion that supercritical CO₂ is more compressible than liquid CO₂ is incorrect. The ranges of compressibilities of the liquid and the supercritical fluids overlap markedly. It is also clear why statements suggesting that the supercritical fluid has properties between those of the gas and the liquid are misleading. Actually the supercritical zone has either gas-like or liquid-like

properties, depending on the operating temperature and pressure and on the property considered. It may be concluded clearly at this point, that sorting operations into two classes, those made under subcritical and those made under supercritical conditions is really not justified and serves no valid purpose from either the instrument design or the operational point of view. The SFC parameters are essentially influenced by the fluid density; so the isopycnic lines can be more effectively used to define the coordinates of the operating regions than the isobar or the isotherm lines.

Operating SFC systems under the experimental conditions found in zone B, however, offers some unique advantages compared to operating them under zone A conditions. The lower density isopycnic lines in zone B (e.g., those with densities between 0.75 and 0.85 g/mL) correspond to pressures much higher than those in zone A. This may be advantageous because operations at high flow rates (hence leading to high pressure drops along the column) can be more conveniently carried out in zone B than in zone A, without leading to outlet conditions being dangerously close to the high compressibility or vapor–liquid equilibrium (VLE) zone. The possibility of operating at relatively low density is quite worthwhile since operating on the 0.85 g/mL isopycnic line rather than on the one at 0.95 g/mL means a near 35% decrease in the mobile phase viscosity and a corresponding decrease in the analysis or separation time. Another important difference between operations in zones A and B is the difference in the operating temperatures. For all the isopycnic lines crossing through both zones, zone B corresponds to higher temperature operations than zone A. As retention factors and column efficiency depend on the solubility, which in turn depends on the temperature and the density [14], the consequence of this difference is important. For example, following an isopycnic line, operations in zone B will lead to a higher solute solubility than operations in zone A. A higher solubility normally translates into a lesser retention, hence faster analyses, if the selectivity can be maintained. For preparative separations, a higher solubility means also a higher potential throughput, hence a higher productivity. This indicates that, if operations in zone A, the subcritical zone, offers several serious advantages, e.g., operational robustness, operations in zone B can have significant performance dividend, mostly without sacrificing the advantages of zone A, provided that the operating conditions are designed carefully.

2.3. Operations in zone C

Operations in zone C are far more challenging than those made in the other two zones defined above. The main problems in this zone originate from the high compressibility of CO₂ (Fig. 3) and the position of the critical density line, which passes through the middle of this zone. The primary consequence of the high compressibility is the large density gradient that may result along the SFC column, even for minor pressure drops. For example at 310 K (Fig. 3), for an inlet pressure of 100 bar and an outlet pressure of 80 bar (20 bar pressure drop), the density falls from 0.65 to 0.25 g/mL. In this range, Bartle et al. [20] reported a decrease of the diffusion coefficient for naphthalene in CO₂ by as much as 50% at a temperature 1 K higher than the critical temperature but only 5% at a temperature 9 K higher. The zone of operational difficulties described by previous publications (see Fig. 2) approximately overlaps this zone C. The isopycnic lines however more clearly brings out the outlines of the troubled region. Further study is required to understand the nature of the problems.

3. Experimental

We made a series of measurements with two PAHs, anthracene and pyrene, to verify the conclusions derived from the discus-

Table 2
Retention factors of anthracene (k_1) and pyrene (k_2).

Temperature (K)	Pressure (bar)	Density (g/mL)	$k_1(-)$	$k_2(-)$
300	280.2	0.95	0.82	1.64
300	193.0	0.9	0.98	2.02
304	223.0	0.9	0.92	1.89
310	262.0	0.9	0.82	1.65
300	135.5	0.85	1.23	2.58
304	159.5	0.85	1.13	2.34
310	193.8	0.85	1	2.05
320	252.5	0.85	0.86	1.7
300	79.70	0.75	1.71	3.7
304	96.40	0.75	1.61	3.45
310	120.7	0.75	1.53	3.21
320	162.7	0.75	1.28	2.61
340	248.3	0.75	0.97	1.88
310	94.3	0.65	1.97	4.24
320	124.3	0.65	1.82	3.83
340	186.2	0.65	1.5	3
360	249.4	0.65	1.17	2.2
310	86.5	0.55	2.23	4.84
320	108.4	0.55	2.27	4.81
340	153.7	0.55	2.21	4.54
360	199.8	0.55	1.87	3.7
310	83.7	0.45	2.3	4.98
320	100.4	0.45	2.72	5.95
340	133.7	0.45	3.14	6.73
360	167.1	0.45	2.94	6.18
310	81.1	0.35	2.36	5.17
320	93.3	0.35	3.11	6.78
340	117.2	0.35	4.8	10.77
360	140.6	0.35	5.37	11.9

sion of the isopycnic lines and confirm observations reported in the literature. Toluene was used for measuring the column void volume. All the compounds were co-injected, at concentrations of around 1 g/mL. The instrument used was a TharSFC system from Waters (Mildford, MA, USA). This instrument is comprised of (1) a fluid delivery module with three parallel reciprocating pumps with heads maintained at 4 °C, (2) an autosampler, (3) an oven, (4) a photo diode array detector (Waters 2998); and (5) an automated back pressure regulator (ABPR). The column used was a 0.46 cm × 15 cm Atlantis C18 column from Waters, packed with 3 μm particles. The mobile phase was neat CO₂ from Air-gas (Knoxville, TN, USA). In all the experiments the flow rate was kept constant at 3 mL/min, to avoid any change in the retention factors caused by a change in the flow rate [21]. According to the manufacturer of TharSFC, to ensure the set flow rate at the specified temperature and pressure, the frequency of piston strokes in the pump is controlled based on the data set within the PID settings, which account for density and compressibility. The operating pressures, temperatures and densities are listed with the retention factors of the two compounds in Table 2.

4. Variations of the retention factors along isopycnic lines

There are relatively simpler relationships between the retention factors of the solutes with the density and the temperature of the mobile phase [14]. This permits the use of the isopycnic plots to estimate the nature of the variations of the retention factor with the operating temperature and the density.

Fig. 3 shows that the density of CO₂ monotonically increases with increasing pressure at constant temperature. This indicates that the solubility of most compounds in CO₂ increases monotonically with increasing pressure, which in turn, implies that the retention factors of those compounds, which are most often con-

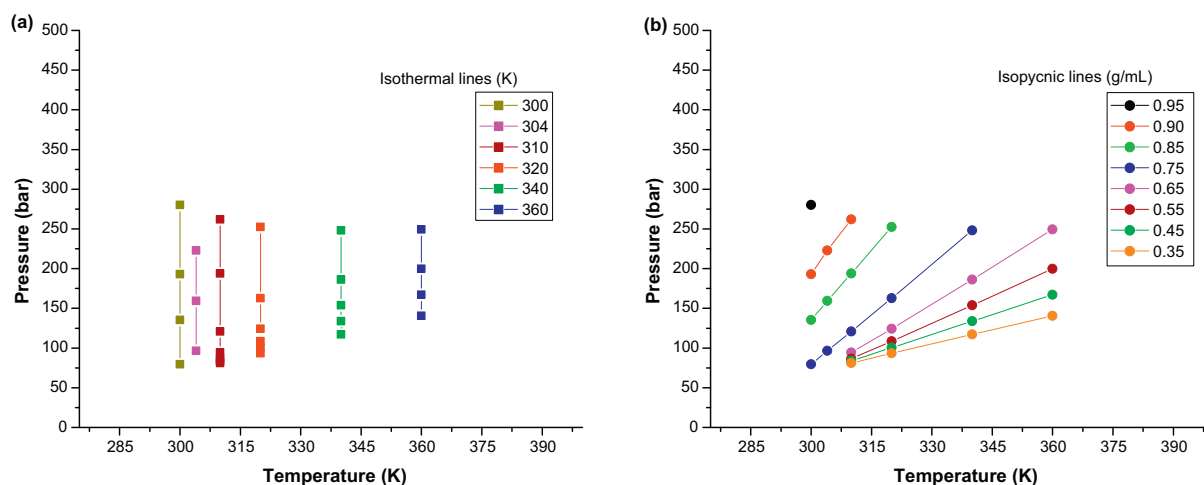


Fig. 4. Operating points considered for the experimental study: (a) connecting isothermal points and (b) connecting isopycnic points.

trolled by their solubility, monotonically decreases with increasing pressure. This insight is useful when designing a set of experimental conditions. However, developing a similar insight regarding the effect of the temperature is far more difficult. Due to the crossover points of isothermal solubilities [22,14], it is impossible to predict whether the solubility of a particular compound will increase or decrease with increasing temperature under isobaric conditions, without specific information on the crossover pressure of that particular compound. This might be a serious disadvantage when the selectivity of certain separations depends much on the operating temperature and some of the compounds involved have a high thermal sensitivity. Using the isopycnic instead of the isobaric lines can help to solve this issue because solubility increases monotonically with increasing temperature along isopycnic lines. So, one can look for suitable operating conditions in terms of either the pressure or the temperature by following either the isothermal or the isopycnic lines, without any need for information regarding the crossover points of the compounds involved.

The experimental results in Table 2 illustrate the application of this understanding. The experimental temperatures and pres-

ures considered for this analysis were chosen to cover a wide range of operating conditions. Experiments were carried out at six different temperatures, starting from 300 K, which is often selected as an operating temperature in subcritical SFC methods. The next temperature chosen was the critical temperature, 304 K. Although rarely employed, the critical temperature was chosen mainly to demonstrate the continuity of the experimental conditions across the critical isotherm. The other temperatures, 310, 320, 340 and 360 K, were chosen to cover a wide temperature range with sufficient intermediate points. The operating pressures at these temperatures (Table 2) were selected to generate mobile phase densities of 0.95, 0.9, 0.85, 0.75, 0.65, 0.55, 0.45 and 0.35 g/mL. The operating pressures and temperatures considered for this study are illustrated in Fig. 4.

The retention factors of anthracene and pyrene corresponding to the experimental conditions shown in Fig. 4 are provided in Figs. 5–7. Fig. 5 shows that at all the temperatures considered in these measurements (see Fig. 4(a)), the retention factors monotonically decrease with increasing pressure, which supports our earlier observation. In the current discussion, however, the main

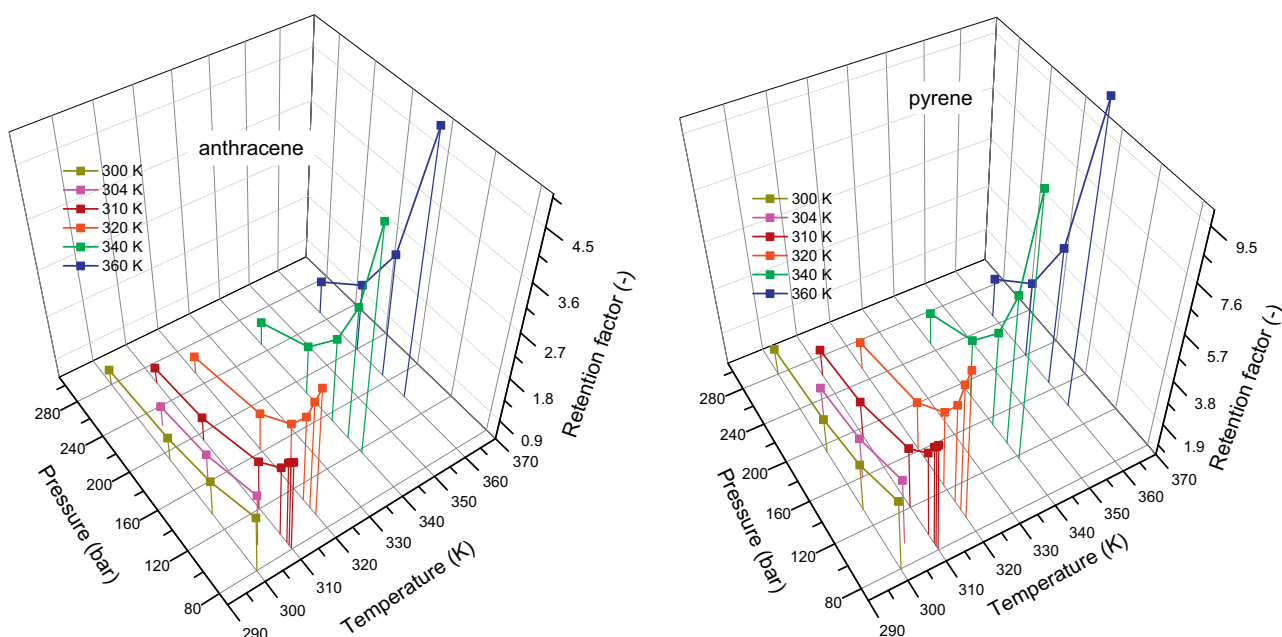


Fig. 5. Variation of the retention factors with pressure at isothermal conditions.

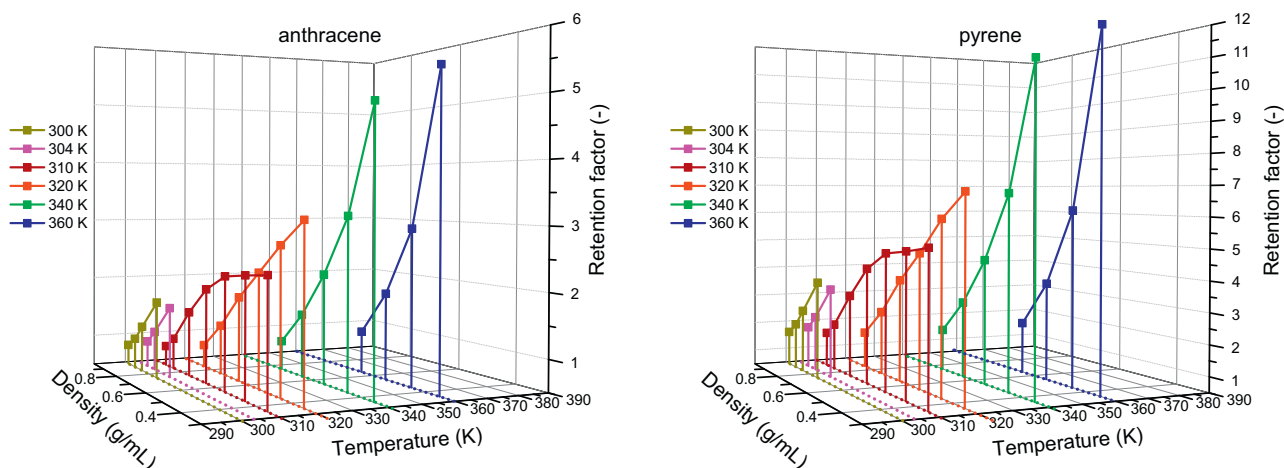


Fig. 6. Variation of the retention factors with density at isothermal conditions.

concern is to understand the change in the retention behavior as functions of the density and the temperature of the mobile phase. To follow these variations, the retention data shown in Fig. 5 are replotted in Figs. 6 and 7. Fig. 6 shows that the retention factors monotonically decrease with increasing density under isothermal conditions, which is not unexpected because increasing the density under isothermal conditions requires increasing the pressure, and retention factors decrease with increasing pressure (Fig. 5). However, reconnecting the same data points shown in Fig. 6 according to their density (Fig. 7) provides an important, interesting insight. It can be noted from Fig. 7 that at densities of 0.65 g/mL or higher the retention factors of both compounds decrease monotonically with increasing temperature, which should be expected because solute solubility increases monotonically under such conditions. However, at densities of 0.55 g/mL or lower, this general trend is no longer valid. At a density of 0.55 g/mL, the retention factors decrease very slowly. In fact, for anthracene, the retention factor seems to increase very slightly from 310 to 320 K, but this change is negligible, considering the experimental error. At a mobile phase density of 0.45 g/mL, the retention factors of both solutes clearly increase up to 340 K, above which temperature they decrease. At 0.35 g/mL the retention factors increase monotonically up to 360 K, which is the highest temperature considered for these measurements. Elution profiles of anthracene and pyrene at mobile phase

densities of 0.75, 0.55 and 0.35 g/mL and at increasing temperatures are shown in Figs. 8–10 respectively for further elucidation.

Such changes in the retention pattern cannot be controlled only by the variations of the solubilities of the test compounds in CO_2 . This can be asserted because (a) the solubility of a compound increases with increasing temperature at constant density, leading to a possible decrease in the retention factor in the current situation, not to an increase; (b) at densities of 0.55 g/mL or lower, the solubility is practically independent of the temperature, as shown in the companion paper [14], so a solubility effect is a highly unlikely explanation of the increase of the retention factors with increasing temperature. Under similar circumstances Lou et al. [23] invoked the possibility that such an unexpected retention behavior could be due to a change in the solute affinity for the stationary phase, rather than a mobile phase solubility effect. However, because the experiments reported in their report were not performed under isopycnic conditions, no direct equivalence can be drawn. Additionally, Lou et al. [23] did not provide a convincing physical explanation of why the solute affinity for the adsorbent should increase with increasing temperature.

In summary, the experimental results generally confirm the expected behavior of the retention factors over wide ranges of temperature and pressure, including parts of both the subcritical and the supercritical regions, provided the operating points are

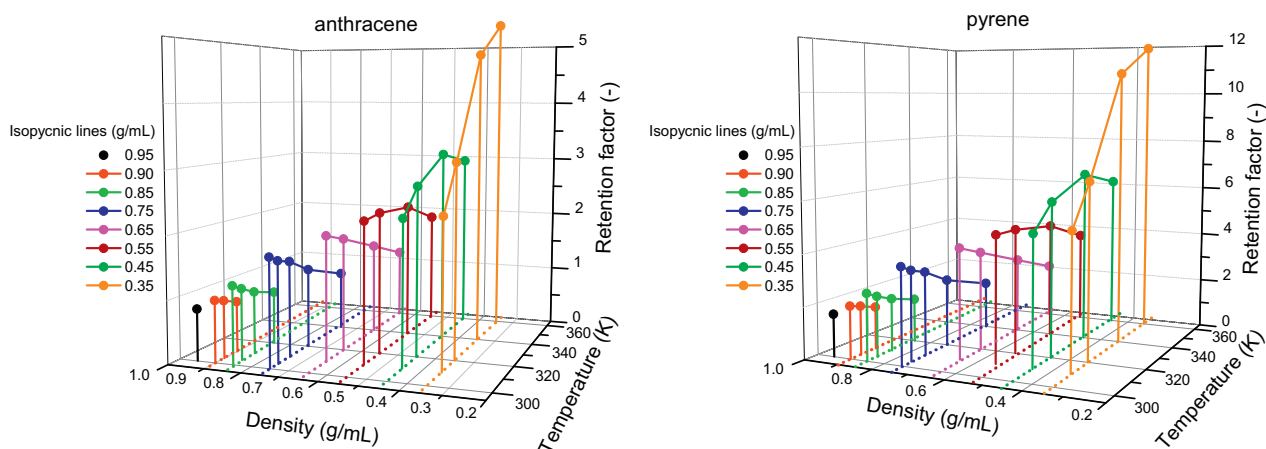


Fig. 7. Variation of the retention factors with temperature at isopycnic conditions.

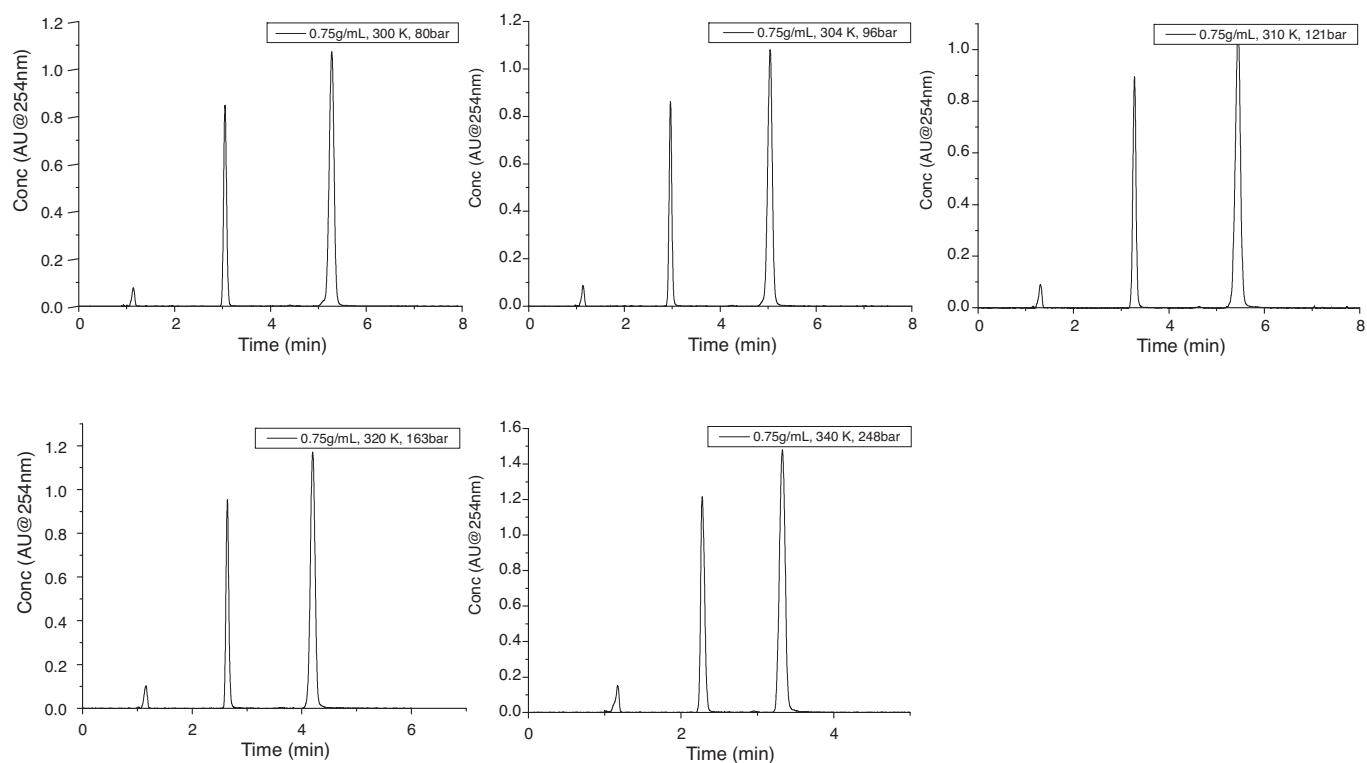


Fig. 8. Elution profiles of anthracene and pyrene at 0.75 g/mL and increasing temperature.

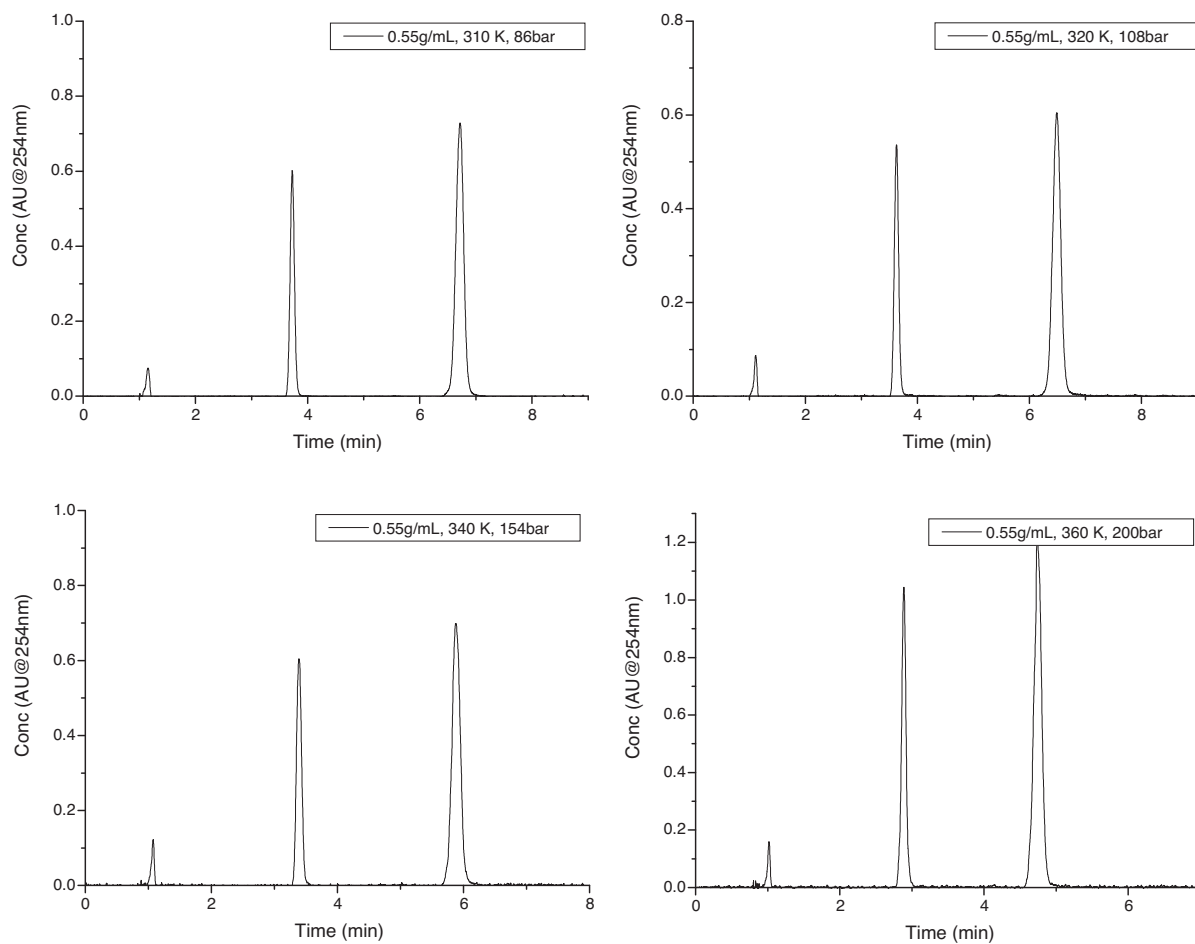


Fig. 9. Elution profiles of anthracene and pyrene at 0.55 g/mL and increasing temperature.

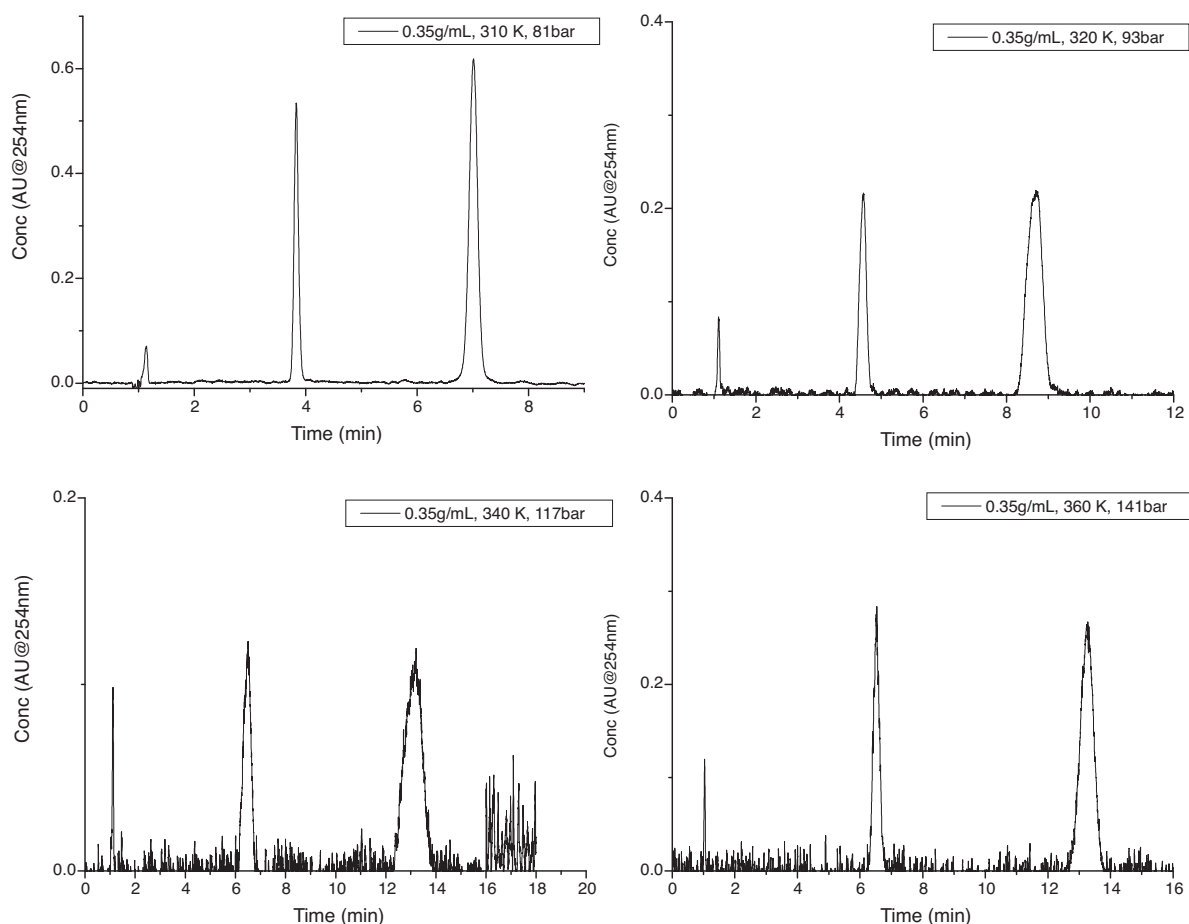


Fig. 10. Elution profiles of anthracene and pyrene at 0.35 g/mL and increasing temperature.

largely within the boundaries of zones A and B. Inside zone C, the retention behavior defies the trend predicted based on the simple observation of the density and the solubility dependence of the retention factors. The experimental results lead to the following observations:

1. These results demonstrate that the critical isotherm cannot provide any useful operational understanding because the variations of the retention factors across the critical isotherm are continuous, as illustrated by the data measured along the isopycnic lines at 0.9, 0.85 and 0.75 g/mL. From an operational point of view, there is no difference between the chromatograms (e.g. Fig. 8) obtained under experimental conditions represented in the pressure–temperature plane by two neighboring operation points depending on whether both points are in the sub- or in the supercritical zones, or whether they are located on either side of the critical isotherm.
2. When the operating pressure is below 150 bar, the separations are better at temperatures close to the critical temperature, higher or lower, than at high temperatures in the supercritical region. For example, one of the points on the 0.75 g/mL isopycnic line, the one with an operating temperature and pressure of 304 K and 96 bar, respectively, is close to the critical point. These operating conditions, however, did not lead to any unwanted noteworthy consequence. The chromatograms do not differ considerably from those obtained under experimental conditions corresponding to neighboring points and the retention factors are intermediate. In contrast, operations in zone C, especially

at densities below 0.65 g/mL, at temperatures markedly higher than the critical temperature, resulted into unwanted consequences. No physical explanation can be provided to explain this behavior in zone C. Further investigations are required. They were not within the scope of this study.

5. Conclusion

This work demonstrates the importance of considering the isopycnic lines when selecting the experimental conditions in SFC because the physical properties of CO₂ that are relevant to the operation of chromatographic systems depend strongly on the eluent density, much more than on the column temperature and pressure. The distribution of the isopycnic lines on the pressure–temperature plane suggests to distinguish three different operating zones in this plane. A discussion of the behavior of CO₂ in these different zones confirms that considering boundaries between the subcritical and the supercritical operations is artificial. A more insightful approach to the design of SFC operations should be based on an analysis of the behavior of the isopycnic lines that cuts through the boundary between the subcritical and supercritical conditions. As the critical isotherm does not provide any insight to the consequences of SFC operation, denominations like subcritical or supercritical fluid chromatography do not carry any practical utility. A discussion of the properties of these lines and of the zones of the pressure–temperature diagram of CO₂ shows that, in a wide region of this diagram, both in the subcritical and in the supercritical state, at pressures higher than

300 bar, the compressibility of CO₂ is rather low, suggesting that there is little difference between liquid chromatography and SFC operated in that region. This also suggests that it could be possible and useful to extend to SFC the design and optimization tools that were successfully developed for HPLC operation, with due adjustment of the algorithms and programs used. The location and the distribution of the isopycnic lines show that while operations in the subcritical zone may lead to more stable conditions, operations in the supercritical zone might bring high operational dividends.

Determining suitable operating conditions in SFC has long been a challenging task. It was already asserted in several publications that crossing the critical pressure and the critical temperature lines does not bring any significant change in the chromatographic behavior and is largely irrelevant. This realization, however, did not help the design task. In this paper, we show that consideration of the isopycnic lines provides a possible road map in estimating the change of chromatographic properties from the pressure–temperature diagram. This should be helpful in designing SFC operations.

Although the most attractive operating conditions in SFC are in the low pressure region, where the viscosity is low and the diffusivity high, hence where analyses could be fast, it is also more difficult to design SFC operations here, due to the steep changes in the eluent density that take place even over small pressure drops. These changes result into significant variations of the mobile phase properties along the column, which explains why this zone of opportunity in SFC is rarely used. Currently, the lack of a detailed understanding of all the consequences of operating in this zone makes it safer to perform separations in zones A and B of the pressure–temperature diagram. Further research on the behavior of CO₂ in zone C is necessary to understand the limits of SFC operations. The use of the isopycnic plots could greatly assist in this endeavor.

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