



Efficiency in supercritical fluid chromatography with different superficially porous and fully porous particles ODS bonded phases

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ABSTRACT

The chromatographic efficiency, in terms of plate number per second, was dramatically improved by the introduction of sub-two microns particles with ultra-high pressure liquid chromatography (UHPLC). On the other hand, the recent development of superficially porous particles, called core-shell or fused-core particles, appears to allow the achievement of the same efficiency performances at higher speed without high pressure drops. CO₂-based mobile phases exhibiting much lower viscosities than aqueous based mobile phases allow better theoretical efficiencies, even with 3–5 μm particles, but with relative low pressure drops. They also allow much higher flow rates or much longer columns while using conventional instruments capable to operate below 400 bar. Moreover, the use of superficially porous particles in SFC could enhance the chromatographic performances even more. The kinetic behavior of ODS phases bonded on these particles was studied, with varied flow rates, outlet (and obviously inlet) pressures, temperatures, by using a homologous series (alkylbenzenes) with 10% modifier (methanol or acetonitrile) in the carbon dioxide mobile phase. Results were also compared with classical fully porous particles, having different sizes, from 2.5 to 5 μm. Superior efficiency (*N*) and reduced *h* were obtained with these new ODS-bonded particles in regards to classical ones, showing their great interest for use in SFC. However, surprising behavior were noticed, i.e. the increase of the theoretical plate number vs. the increase of the chain length of the compounds. This behavior, opposite to the one classically reported vs. the retention factor, was not depending on the outlet pressure, but on the flow rate and the temperature changes. The lower radial trans-column diffusion on this particle types could explain these results. This diffusion reduction with these ODS-bonded superficially porous particles seems to decrease with the increase of the residence time of compounds.

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1. Introduction

In the race to achieve high efficiency in the shortest analysis time, fully porous sub-two microns particles and superficially porous particles seem to compete comparatively. However, while the former induce ultra-high pressure drops limiting the possible column length, the latter can reach high chromatographic efficiency with classical pressure drops, allowing the use of longer column lengths.

Superficially porous particles were introduced in the late 1960s by pioneers, either with ionic pellicular phases [1,2] or with controlled surface porosity supports for GC [3], for LC [4] or for both [5]. These first studies showed the dramatic reduction in the *C* term of van Deemter curves, allowing the achievement of very good efficiency performances at higher flow rates, despite the large particle diameter, around 50 μm. Reduced HEPT (*h*) as low as 2.7 was

reached [5]. Following these first attempts, superficially porous particles with a 5 μm solid core coated by two shells of 0.5 μm thickness (1 μm total) were developed [6]. However, the final particle distribution was a little large, from 5.5 to 10 μm, and the pore diameter was around 300 Å. Higher performance of these phases was reported mainly for large molecules. Recently, the same authors reported the development of silica microspheres composed of a solid core and thin outer shells with varied particle diameters and shell thickness [7,8]. A reduced plate height of 2 was achieved with these particles in water-rich mobile phase [7].

Another study underlined that the *C* term was close between a porous particle with a pore size of 300 Å and the superficially porous Poroshell 300-SB, indicating almost identical mass transfer properties, but the *A* term was lower on the second, thanks to a better packing [9]. Moreover, the use of a column length equal to 60 cm allowed achieving higher peak capacity. The peak capacity per unit pressure drop or per unit time of the pellicular materials is very favorable, allowing the separation of complex mixtures such as BSA tryptic peptides [9]. However, even with pellicular particles, low flow rates (below 0.5 ml/min) and high temperature (around

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Table 1
Column length, pressure drop and methylene selectivity of some phases used in SFC.

Column name	Size (mm)	Particle diameter micron	Pore diameter (Å)	Surface area (m ² g)	Carbon load (%) of surface coverage	Type
Luna C18(2)	250 × 4.6	5	100	400	17.5	Fully porous
Luna C18(2)	100 × 3	2.5	100	400	17.5	Fully porous
HSS C18	150 × 4.6	5	100	230	15	Fully porous
Zorbax eclipse plus	250 × 4.6	5	95	160	9	Fully porous
Strategy C18-3	150 × 4.6	3	100	425	22	Fully porous
Pursuit UPS	100 × 3	2.4	100	350	21	Fully porous
Halo C18	150 × 4.6	2.7	90	150	3.7 μmol/m ²	Superficially
Halo Peptide ES-C18	150 × 4.6	2.7	80	160	2.0 μmol/m ²	Superficially
Kinetex C18	150 × 4.6	2.6	100	200	12	Superficially
Kinetex XB-C18	150 × 4.6	2.6	100	200	10	Superficially
Poroshell 120 EC-C18	150 × 4.6	2.7	120	120	8	Superficially

70 °C) seem required to maintain pressure drop in a reasonable range when working with liquid mobile phases.

Supercritical fluids display a lower viscosity than liquids (from 1/15th to 1/40th [10]). As a result, in supercritical fluid chromatography (SFC), higher flow rates can be used than in HPLC [11–13]. Moreover, the lower viscosity enhances the diffusion of compounds in the mobile phase, leading to a shift of the optimal velocity towards these higher flow rate values, around 3 ml/min [13]. However, these values depend on the fluid density and on the modifier amount.

In addition to these diffusion properties at high flow rates, the high eluting power of CO₂/modifier mixtures [14], also improves fast analyses, explaining an increase of the use of SFC, both in normal and reversed-phase mode [15–17] and for varied applications [18–22]. The combination of these fluids with superficially porous particles would provide high separation performances, and is the topic of this paper. Recently, in SFC, superficially porous (2.6 μm) and fully porous silica (3 μm) particles were compared, showing the higher efficiency per unit time of superficially porous particles [23]. These results were obtained with an apparatus that was optimized for SFC allowing very low extra-column dispersion.

In this paper, a comparative study is performed on ODS phases, which can also be used in SFC, allowing to cover not only the normal phase mode domain, as often described by numerous authors, but also the reversed one, as described elsewhere by numerous studies on column interactions [24–28].

2. Materials and methods

Chromatographic separations were carried out using modified HPLC equipment. Two model 980-PU (Jasco, Tokyo, Japan) pumps were used, one for carbon dioxide and a second for the modifier. Control of the mobile phase composition was performed by the modifier pump. The pump head used for pumping the carbon dioxide was cooled to –5 °C by a cryostat (Julabo F10c, Seelbach, Germany, supplied by Touzart et Matignon, les Ulis, France). When the two solvents (methanol and CO₂) were mixed, the fluid was introduced into a dynamic mixing chamber PU 4046 (Pye Unicam, Cambridge, UK) connected to a pulsation damper (Sedere supplied by Touzart et Matignon).

The injector valve was supplied with a 20 μL loop (model 7125 Rheodyne, Cotati, CA, USA). The tubing between the injection valve and the column was 15 cm × 0.18 mm (ID). For partial loop injections (from 1 to 15 μL), the loop contains only the injected volume. Manual starts were performed. To ensure no thermal difference between the mobile phase and the column, 50 cm stainless steel tubing (0.18 mm ID) is located inside the oven, between the dynamic mixing chamber and the injector.

The columns were thermostated by an oven (Jetstream 2 Plus, Hewlett-Packard, Palo Alto, USA). The detector was a UV–vis Gilson

151 equipped with a cell having a path length equal to 5 mm; volume of 10 μL. The detection wavelength was 210 nm. After the detector, the outlet column pressure was controlled by a Jasco 880-81 pressure regulator (supplied by Prolabo). The outlet regulator tube (internal diameter 0.25 mm) was heated to 60 °C to avoid ice formation during the CO₂ depressurization. The apparent void volume was measured from the first negative deviation of the signal. To ensure the proper calculation of V₀ from this negative deviation, the porosity of the Zorbax Eclipse plus (250 mm × 0.46 mm) was calculated. With a t₀ equal to 0.91 min, at a flow rate of 3 ml/min, leading to a void volume of 2.73 ml and a total porosity equal to 0.66, which is in accordance with most of the values encountered in the literature for these particle and column dimensions. This indicates that despite the density variation of CO₂ due to temperature change between the pump (at –5 °C) and the column (25 °C), and despite the low retention factor values (between 0.6 and 2.8), the relevance of *k* calculation seems warranted in this case. However, the same calculation for Kinetex leads to an apparent total porosity of 0.66, which seems higher than the one classically reported (around 0.54). This is often encountered with the use of the SFC compressible fluid, because of higher density related to higher inlet pressure. At identical flow rate and backpressure, the inlet pressure is higher for superficially porous particles of 2.6 μm than for 5 μm fully porous particles. The fluid volume required to fill the void volume is thus larger. As a result, the apparent void volume is larger, thus the calculated porosity is larger. Besides, this density increase also enhances the eluting strength of the mobile phase, thereby reducing the retention time. These two phenomena could lead to reduction of the calculated retention factor. However, the perfect straight line (*R*² = 0.9996) for log *k* vs *n*_C (carbon number) for the 9 alkylbenzenes studied on Kinetex C18 (not shown), as expected when working with homologous series, shows the relevance of the retention factor calculation. What is more, the close values of methylene selectivity (presented in Table 2) calculated from the slope of the log *k* vs *n*_C regression line, seems in accordance with this statement for all columns.

Column characteristics are summarized in Table 1. For superficially porous particles, columns used were: Kinetex C18 (150 mm × 4.6 mm; 2.6 μm), Kinetex XB-C18 (150 mm × 4.6 mm; 2.6 μm), Luna C18(2) (250 mm × 4.6 mm; 5 μm) and Luna C18(2) (100 mm × 3 mm; 2.5 μm) (Phenomenex, Le Pecq, France); Halo C18 (150 mm × 4.6 mm; 2.7 μm); Halo peptide ES-C18 (150 mm × 4.6 mm; 2.7 μm) (Mac Mod, Chadds Ford, USA); Poroshell 120 EC-C18 (150 mm × 4.6 mm; 2.7 μm) (Interchim, Montluçon, France). For fully porous particles, the columns were: HSS C18 (150 mm × 4.6 mm; 5 μm) (Waters, Cergy Pontoise, France), Zorbax Eclipse plus (150 mm × 4.6 mm; 5 μm) (Agilent, Massy, France) Strategy C18-3 (150 mm × 4.6 mm; 3 μm) (Interchim); Pursuit UPS (100 mm × 3 mm; 2.4 μm) (Varian, les Ulis, France). The mobile phase was CO₂/methanol or CO₂/acetonitrile

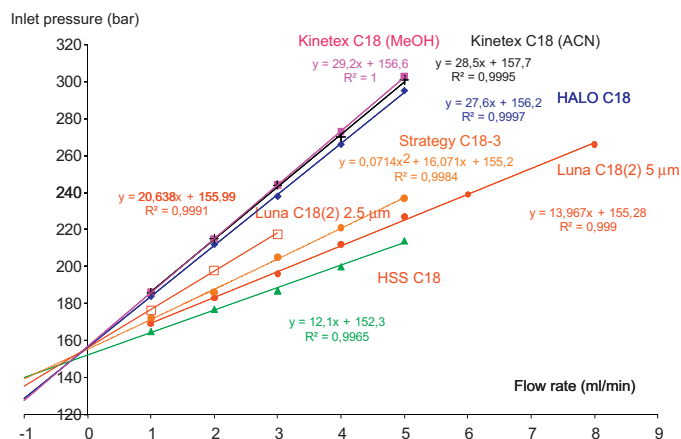


Fig. 1. Inlet pressure vs. flow rate for different columns. Mobile phase: CO₂/MeOH 90/10 (v/v) 25 °C; outlet pressure = 15 MPa. Open red squares: Luna C18(2); 2.5 microns (10 cm × 3 mm) (data for a column diameter equal to 4.6 mm are calculated from experimental values obtained with a column diameter equal to 3 mm); pink full squares: Kinetex C18; 2.6 microns (15 cm) (modifier methanol); black crosses: Kinetex C18; 2.6 microns (15 cm) (modifier acetonitrile); blue diamonds: Halo C18; 2.7 microns (15 cm); orange full circles: Strategy C18-3; 3 microns (15 cm); red full small circles: Luna C18(2); 5 microns (25 cm); green full triangles: HSS C18 5 microns (15 cm). (For interpretation of the references to color in this figure caption, the reader is referred to the web version of the article.)

(90/10, v/v); injected volume was 1 μl per 5 cm of column length; column temperature was 25 °C; outlet pressure was 15 MPa. A 9 alkylbenzene mixture (with carbon number ranging from C11 to C19) was injected diluted in ethanol/methylene chloride. The chromatograms were recorded by the Azur software (Datallys, St Martin d'Heres, France), and the theoretical plate number was calculated by this software from the peak width measured at half height.

3. Results and discussion

3.1. Pressure drop and theoretical plate number

Fig. 1 shows the inlet pressure vs. flow rate for six columns (among the eleven studied columns) with different particle sizes and types. All pressure variations are constant (the curves all fitted well by a linear regression), showing the relevance of the pumping system and of the back pressure regulator. The intercept of the curves is close to 15 MPa, which was the selected outlet pressure. However, in order to compare the pressure drop with equal column length, the values are also expressed per ml and per cm of column length (Table 2).

As expected, the two 5 μm particle phases (Luna C18(2) and HSS C18) display the lower pressure drop per ml per cm (0.56 and 0.8 bar). The higher value obtained for HSS can be explained by the lower permeability of this stationary phase, as indicated by the porous volume, equal to 1 ml/g for Luna C18(2) and to 0.7 ml/g for HSS C18.

The inlet pressure values of the superficially porous phases of 2.6 and 2.7 μm (Kinetex C18 and Halo C18) are higher than the one of the 3 μm fully porous phase (Strategy C18-3), as expected from the particle diameter values, but slightly lower than the one obtained

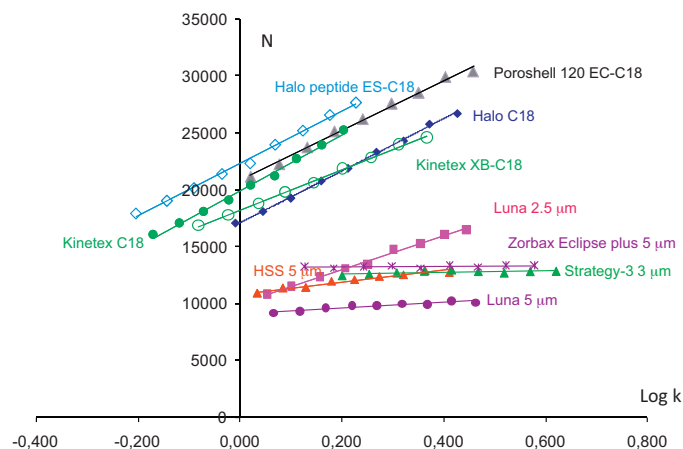


Fig. 2. Experimental theoretical plate number for alkylbenzenes (C11–C19) vs. log *k* for different columns (symbols as Fig. 1). Same mobile phase, outlet pressure and temperature as in Fig. 1, flow rate of 3 ml/min for 4.6 mm and of 1.0 ml/min for 3.0 mm column.

for the Luna C18(2) 2.5 μm fully porous particle phase. Naturally, the packing quality and the particle size distribution also have an influence on pressure drop.

Moreover, these values of pressure drop per ml per cm are dramatically lower than the ones obtained in HPLC due to the lower viscosity of the supercritical mobile phase, despite the addition of 10% methanol to carbon dioxide. It explains that the superficially porous particles can be used with a flow rate equal to 5 ml/min without reaching a higher inlet pressure than 300 bar (thus allowing the use of classical pumping systems). As a result, with 5 μm fully porous particles, flow rates larger than 10 ml/min can be easily achieved in these SFC conditions.

Besides, when comparing the two superficially porous particles, Kinetex and Halo, and as found by Gritti and Guiochon with pure acetonitrile and acetonitrile/water 80/20 (v/v) mobile phases [29], the inlet pressure is slightly higher on Kinetex in regards to Halo, due to the lower specific permeability *k*₀.

$$k_0 = \frac{\eta L}{\pi R_c^2 (\Delta P / F_v)} \quad (1)$$

With liquids, the ratio of the two specific permeabilities (Halo/Kinetex) increases with the mobile phase viscosity from 1.15 to 1.19. Because the specific permeability is inversely related to the ratio between the pressure drop and the flow rate ($\Delta P / F_v$) (Eq. (1)), the specific permeability ratio (*k*₀₂/*k*₀₁) calculated for two columns with the same mobile phase is equal to the ratio of the slopes of the ΔP vs. flow rate curves. With the CO₂/methanol mobile phase employed here, the ratio is equal to 1.05, showing the lower difference in the specific permeability between the two superficially porous phases with supercritical fluids, due to the lower mobile phase viscosity.

The methylene selectivities obtained for the studied columns are very close (Table 2). The values are strongly lower than the values obtained in HPLC showing the strong eluting power of the modifier/CO₂ mobile phase.

Table 2

Column	HSS C18	Luna C18(2)	Strategy C18-3	Halo	Kinetex C18	Luna C18(2) 2.5
<i>L</i> (cm)	15	25	15	15	15	10
ΔP (bar)/ml	12.1	14	16.5	27.6	29.2	21
ΔP (bar)/ml cm	0.8	0.56	1.1	1.84	1.94	2.1
α CH ₂	1.114	1.119	1.128	1.113	1.114	1.121

Fig. 2 shows the plate number values (N) of alkylbenzenes (C11–C19) for different columns vs. the logarithm of the retention factor ($\log k$). The plate number values are expressed for columns having the same length equal to 15 cm. For these measurements, the flow rates were equal to 3 ml/min for all phases except for Luna C18(2) 2.5 μm (1 ml/min). This change is related to the smaller internal diameter of this column (3 mm against 4.6 mm for all others), to ensure close linear speed of the mobile phase for all columns.

Several main points can be discussed from this figure.

First, the plate number of fully porous particles (calculated from 15 cm column length) varies from 10,000 (Luna C18(2) 5 μm) to 16,000 (Luna C18(2) 2.5 μm). Depending on column efficiency, 3 and 5 μm particle columns can present identical plate number values. The reduced h values calculated from these plate number values vary between 2.3 for Zorbax Eclipse plus 5 μm and 3.8 for 2.5 μm (Luna C18(2)) or 3 μm (Strategy C18-3) particles, or 5 μm (Luna C18 (2)). Some of these values seem rather classical [11,12,29], and not very different from HPLC ones [29]. However, as expected from theory, a lower h value of 2.7 was reported on a 3 μm ODS phase using naphthalene as probe in SFC [30], showing the mediocre value observed on the studied phase in the current study. A value of 2.2 was also reported on 3 μm silica particles [23]. It underlines that the comparison of reduced h is sometimes difficult from SFC to SFC because of the different analytical conditions, pure CO_2 or CO_2 -modified mobile phase, temperature, inlet pressure, which lead to different density and diffusion coefficient. Moreover, the column filling quality also impacts the results.

Secondly, the superficially porous particles exhibit higher plate number values, in regards to the other particle sizes and types. The plate number on the superficially porous C18 particles is twice that of fully porous particles, showing the great interest in using these ODS phases in SFC, as previously reported for silica particles [23]. The narrow distribution of particle size for these columns, leading to a reduced eddy dispersion, was supposed to be involved in the greater efficiencies observed for these phases [29,31]. Maximal values are above 25,000 plates, both for Halo (C18 and ES) and Kinetex (C18 and XB-C18, and a little higher for Poroshell (above 31,000). At this flow rate (3 ml/min), which may not be the optimal one, this plate number value (25,000) yields a reduced HETP (h) around 2.2 (considering a particle size equal to 2.7 μm), which remains higher than the extremely low values obtained in HPLC ($h = 1.2/1.4$) with acetonitrile-rich mobile phases [29,31] and in SFC on silica ($h = 1.6$ at 50 °C). Besides, using fully porous particles of 1.8 μm , a h value equal to 2.5 was achieved in SFC with CO_2 /methanol phases with 8% methanol, with a backpressure equal to 15 MPa and a temperature of 50 °C [10]. The optimal plate number was equal to 22,500 by using a column of 100 mm \times 3 mm, which is a little lower than the value reported for Halo and Kinetex 150 mm. However, the stationary phase was also pure silica for the sub-two microns phase [10], instead of alkyl-bonded silica in our study. In both cases, and in addition to different analytical conditions for the compared studies, the different surface chemistry could induce different exchange kinetics between the mobile and the stationary phase, leading to varied h values. Moreover, in terms of dispersion, the apparatus performance also impacts the optimum h values.

The retention on Kinetex C18 is lower than the one on Halo C18. This is in accordance with the lower surface area of Kinetex, equal to 100 m^2/g , against 150 m^2/g for the Halo, due to the lower thickness of the superficially porous area (0.35 μm for Kinetex against 0.5 for Halo).

Kinetex XB-C18, which has butyl side chains, and Poroshell 120 EC-C18 (130 m^2/g ; 8% carbon; 0.5 microns thickness for the superficially porous area) display close retention to Halo C18, whereas Halo ES C18 (160 Å ; 80 m^2/g) is rather similar to Kinetex C18. The lower retention of Halo ES C18 in regard of the one of Halo C18 is explained by a lower surface coverage for Halo ES C18 (2.0 $\mu\text{m}^2/\text{m}^2$

vs 3.7 $\mu\text{m}^2/\text{m}^2$), which is due in part to the steric hindrance of isobutyl size groups on Halo ES. Besides, efficiency on Halo ES C18 seems slightly higher than the one on Halo C18, in accordance with other results [32], whereas the efficiency for the two Kinetex is close.

The last important point in Fig. 2 is the slope of the N vs. $\log k$ straight lines. The slope is zero (or close to 0) for the 3 and 5 μm phases, positive for the smaller fully porous particles Luna 2.5 (and also for Pursuit 2.4 μm , not shown on Fig. 2), and strongly positive for the five superficially porous ones. These positive slopes mean that efficiency increases with retention, thus is larger for the largest alkylbenzenes. This plate number variation, related to the retention time of compounds, was not reported in SFC with silica Kinetex [23], despite an unexpected peak fronting which was observed in comparison with fully porous Luna silica. In this case, the silica acidity difference could induce these varied kinetics.

The low retention factors of the first alkylbenzenes could also explain this difference, because extra-column effects are proportionally more significant for the less retained compounds [23]. However, as seen in Fig. 2 (i) the retention factors on Halo, Kinetex and Poroshell 120 EC-C18 are close to the ones measured on most of the fully porous particles, which do not display the surprising increase of plate number with the alkylbenzene chain length, (ii) a linear change of N vs $\log k$ is observed, rather than the classical asymptote observed for extra-column effects [23], (iii) the total extra column volume, measured by connecting the capillary without column by injection of 3 μl of pure methanol in SFC conditions (3 ml/min; 25 °C, $P = 20$ MPa), was minor that 20 μl and represents only around 1% of the hold-up volume of the partially porous particles.

3.2. Injected volume and inlet pressure

Before address a discussion about this surprising behavior, we should try to summarize some knowledge on efficiency. However, we should also be aware that most of these knowledges are obtained from reduced h values, whereas we present H data in this paper. The reason is that the calculation of reduced linear speed (v) required the solute diffusion coefficient D_m , which is not available in CO_2 /MeOH mixtures. Column dispersion phenomena are complex, depending on the axial diffusion (B term), the eddy dispersion (A term), the trans particle (C_p) and the film mass transfer resistance (C_f) (C term) [32].

The reduced h can be written by this relationship [33,34]:

$$h = \frac{2D_{\text{eff}}}{v}(v) + h_{\text{eddy}} + C_p v + C_f v = B/v + A + C v \quad (2)$$

D_{eff} being the axial diffusion coefficient, related to D_m , h_{eddy} (eddy dispersion) being the sum of transchannel, short and long range interchannel, and transcolumn dispersion, C_p being the transparticle coefficient, related to $1/D_p$, D_p being the particle diffusivity equal to:

$$D_p = D_{\text{pore}} + \rho_p K D_s, \quad (3)$$

with D_{pore} the pore diffusivity, i.e. the solute diffusion into the stagnant mobile phase, ρ_p the particle density, K the retention equilibrium constant, and D_s the surface diffusion coefficient which depends on the isosteric heat of adsorption Q_{st} .

Other equation shows that:

$$D_p = \Omega_p D_m \quad (4)$$

with Ω the relative particle diffusivity to bulk diffusion, Finally, C_f , the film solid-liquid mass transfer coefficient, is related to $1/k_f$, k_f being the film mass transfer coefficient.

Based on some alkylbenzenes (from ethyl to amyl), it has been shown that the diffusion coefficient D_m reduced when chain length

increased, due to the increase of the compound size inducing a decrease in the D_{eff} coefficient in the axial (B) diffusion term (Eq. (2)), which should favor an increase of h , i.e. a large theoretical plate numbers [32].

On the other hand, following the increase in the chain length of alkylbenzene, the relative particle diffusivity to bulk diffusion (\mathcal{D}) [33] was increased (Eqs. (4) and (3)), showing a greater increase of D_p whatever D_m [33]. This increase is supposed to reduce the trans-particle dispersion (C_p), i.e. the resistance to mass transfer between the moving and the stationary mobile phases.

Nevertheless, the total C term increases, from ethyl to amyl benzene, because the film mass transfer coefficient (k_f), decreases, leading to the increase of the C_f term. Consequently, for flow rates reducing the axial diffusion phenomenon (B term), the reduced HETP decreases from ethyl to amylbenzene, the compounds used in the referenced study [33].

In accordance with the pore diffusivity changes reported in Ref. [33], other works [35–39] show that D_{pore} (Eq. (3)) increases for larger alkylbenzenes, that would lead to the increase of D_p , favoring the increase in the theoretical plate number for these alkylbenzenes.

However, the contribution of surface diffusion ($\rho_p k D_s$) accounts for 80–90% in the particle diffusivity (D_p), whereas the pore diffusivity explains only the remaining 10–20%. Moreover, these studies underline that the surface diffusion D_s is lower for larger alkylbenzenes, inducing greater band broadening for the longer retained alkylbenzenes, due to a greater isosteric heat of adsorption of the higher alkylbenzene.

In summary, the surface diffusion (D_s) seems to rule the C term, and should favor narrow peaks for the first eluted alkylbenzenes in regards to the more retained. In accordance with these statements, other studies carried out in HPLC, using Kinetex C18 and Halo C18 columns, have shown that the minimal reduced HETP is lower (greater theoretical plate number) for the less retained compounds (anthracene vs. naphtho[2,3-*a*]pyrene) [40].

However, these conclusions are opposite to the behavior observed in our study with fused-core particles in SFC.

Besides, in SFC, an additional effect on the plate number was reported, related to the depressurization of CO_2 along the column, which induces a cooling of the column from the inlet to the outlet. This cooling induces a “negative” radial gradient of temperature, the temperature at the center of the column being lower than the temperature near the column wall, leading to drastic efficiency losses for retained compounds [41–44]. This radial temperature gradient depends on the pressure drop along the column, i.e. on the flow rate, the column length and diameter, and the particle size [45], and probably the type of phase, because of the conformational change of the C18 chains, i.e. the diffusion inside the stationary phase, in relation with temperature. Indeed, the higher the oven temperature, the greater is the radial temperature gradient. Our experiments were performed at 25 °C and 3 ml/min to minimize this effect [45]. However, if this effect occurs, it should decrease the efficiency for the most retained compounds whereas the contrary is observed, meaning that it could not be related to our observations on efficiency variations for alkylbenzenes.

The injected volume can also induce changes in peak width, depending on the shell thickness [23,46]. The volume limit depends on the phase capacity, i.e. on the specific surface area which is reduced on superficially porous phases.

Fig. 3a shows that from 1 to 5 μl injected, the plate number values are rather constant on the Zorbax Eclipse Plus 5 μm , whereas for 10 μl injected, the plate number strongly decreases for the least retained compounds.

For the Kinetex phase (Fig. 3b), the results are unchanged up to 3 μl , showing that the positive slope observed was not due to the size of the injected volume (which was equal to 3 μl on Fig. 2).

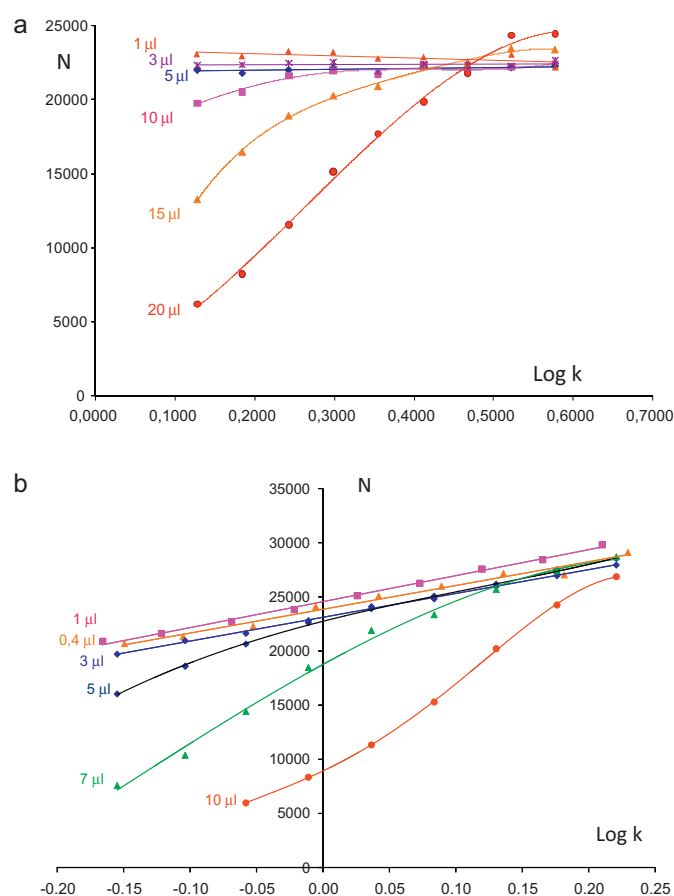


Fig. 3. Effect of the injected volume on theoretical plate number. (a) Zorbax Eclipse plus (250 mm \times 4.6 mm; 5 microns); (b) Kinetex C18 (150 mm \times 4.6 mm; 2.6 microns). Same mobile phase, outlet pressure and temperature as in Fig. 1.

For higher injected volumes, the decrease in theoretical plate number is also observed, due to extra column dispersion. Besides, this limit value of 3 μl seems higher than the one observed on Kinetex silica [23], showing a likely higher phase loadability with ODS phases. Other recent study [47] shown that no efficiency change was reported for compounds having retention factor from 1 to 7, with 10 μl injected on a 4.6 mm (internal diameter Kinetex C18, by using an LC instrument displaying a very low extracolumn variance.

Consequently, all experiments and measurements performed seem conclude that the extra-column effects are not responsible for the observed behavior, i.e. the increase of N for the higher alkylbenzene.

Because the average fluid density varies in SFC with the outlet pressure and the flow rate, and obviously the mobile phase viscosity, i.e. the diffusion phenomena, higher outlet pressures were also studied, from 150 to 210 bar (Fig. 4). Fig. 4a shows no significant changes both on values and on slopes for the plate number measured at 3 ml/min for two phases having fully porous particles of 3 μm (Strategy) and 5 μm (HSS) diameter.

As observed for fully porous particles, for the two superficially porous phases (Kinetex C18 and Halo C18) (Fig. 4b), at 3 ml/min the outlet pressure change does not modify the results, whereas at a lower flow rate (2 ml/min) studied for Kinetex, the pressure increase slightly improves the plate number values, but does not modify the slope. A backpressure increase causes an increase in mobile phase viscosity, thereby reducing the diffusion coefficients (D_m) of the compounds in the mobile phase.

As a result, column efficiency should decrease when pressure increases. However, at 2 ml/min, the fluid density is lower than at

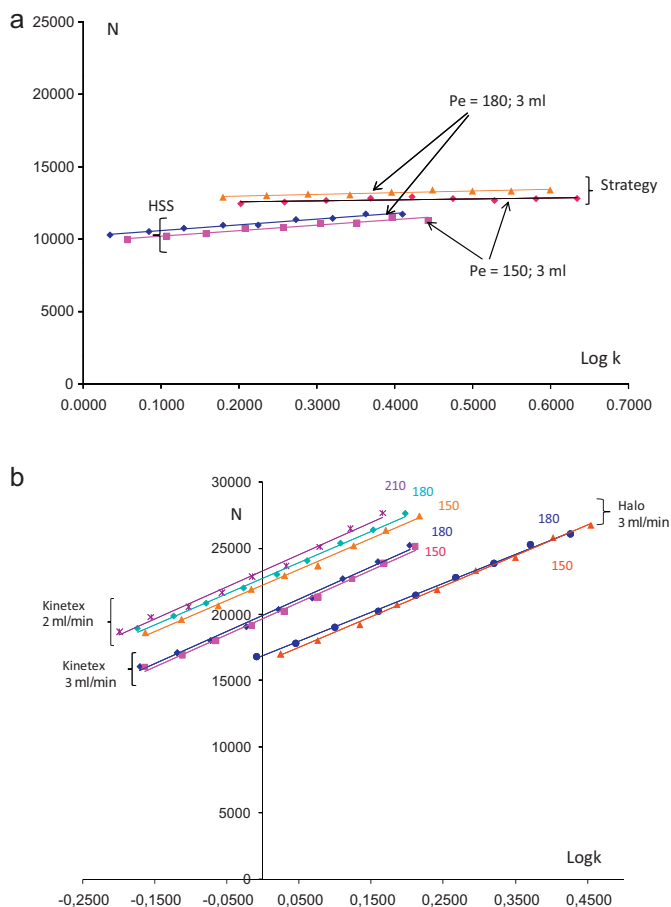


Fig. 4. Effect of outlet pressure (150, 180 and 210 bar) on theoretical plate number. (a) Strategy C18-3 (150 mm \times 4.6 mm; 3 microns); HSS C18 (150 mm \times 4.6 mm; 5 microns); (b) Halo and Kinetex at 2 and 3 ml/min. Same mobile phase, outlet pressure and temperature as in Fig. 1.

3 ml/min. With identical outlet pressure, the lower densities induce a larger density gradient along the column, i.e. a greater linear speed variation, which can reduce the total efficiency. Consequently, at 2 ml/min, the increase in efficiency could be related to the density gradient reduction due to the higher outlet pressure. Nevertheless, these changes are minor, and may not be significant. Besides, the pressure increases favor the retention factor decreases, due to the improved eluting strength of the mobile phase.

3.3. Flow rate modifications

Because of the well-known relationship between HETP and flow rate, this parameter was also studied from 1 to 8 ml/min. Obviously, the range of flow rate achieved in SFC is unusual in regards to the one studied in HPLC, or in UHPLC.

As expected for this chromatographic method [13], for the Luna C18(2) 5 μ m, the increase of flow rate from 1 to 3 ml/min decreases the HETP, whereas the increase from 3 to 8 ml/min increases the HETP value (Fig. 5), i.e. reduces the chromatographic efficiency.

For the Kinetex C18 and Halo C18 (Fig. 6), the optimum flow rate is lower, at 2 ml/min, the higher theoretical plate number being equal to 27,500, i.e. h values equal to 2.1. This optimum flow rate value is close to the one obtained for Kinetex silica in SFC [23], whereas a lower optimum value, around 1.5 ml/min, was also reported in SFC with fully porous 1.8 μ m particles in SFC, but with a lower column internal diameter (3.0 mm) [10]. No significant difference was observed between the two columns (Halo and Kinetex), but the use of acetonitrile instead of methanol favors the efficiency.

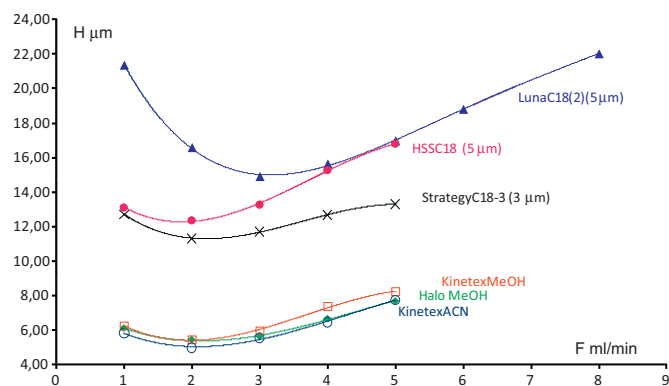


Fig. 5. Variation of H vs. flow rate (F) for nonadecylbenzene on different columns (see legends on figure).

The lower optimal flow rate for these two superficially porous phases shows a slight increase in the C term above 2 ml/min. As expected, this term more strongly increases for higher flow rates with the 5 μ m fully porous particle columns (Luna C18(2); HSS C18). However, the optimal flow rate for the HSS C18 5 μ m was also equal to 2 ml/min, showing no clear relationship between the particle size or geometry and the optimal flow rate value. Besides, the shape of the curve observed for the 3 μ m fully porous particles is close to the one of the superficially porous 2.6 and 2.7 μ m particles, but with a lower C term increase in regards to the one observed on the two fully porous 5 μ m particles HSS and Zorbax. However that may be, this shows that higher flow rates could be used with smaller (fully or superficially porous) particle sizes in SFC. The upper flow rate tested with the superficially porous particles was related to a limit inlet pressure of 300 bar.

On the other hand, for Luna C18(2) 5 μ m, no significant change in the slope of the N vs. carbon number (n_c) curve (Fig. 6a) is observed for different flow rates meaning that no different behavior occurs related either to fluid density modification, i.e. to diffusion coefficient of the compounds, or to their retention times.

Similarly to the curves obtained with the fully porous particles (Fig. 6a), the slope of N vs. n_c for the superficially porous particles is almost constant from 2 to 5 ml/min (Fig. 6b and c).

However, the slope of the curves for Halo and Kinetex is lower at 1 ml/min (Fig. 6c and b), showing that the decrease in mobile phase velocity reduces the previous differences in efficiency between large and small alkylbenzenes.

In accordance with previous works [29,31], and among the numerous phenomena which occur in superficially porous particles, the increase in the residence time of compounds in superficially porous particles, achieved for lower flow rates, could favor the relaxation of the radial concentration gradient across the column diameter, which is due to the velocity difference between the center and the wall of the column tube. This eventually better relaxation allows to reduce the trans-column contribution ($\omega_{B,C}$) to the eddy dispersion (A term in Eq. (2)), that leads to increase the theoretical plate number for the less retained compounds, which are more sensitive to this contribution. This phenomenon could depend both on the type of particle and on the particle diameter, as it is also observed on the 2.4 Pursuit (not shown) and Luna 2.5 microns fully porous particles (Fig. 2). Despite the fully porous nature of particles for Luna C18 2.5, it was reported that the packing of Luna C18(2) is more radially heterogeneous than other ones [48]. As reported elsewhere, this relaxation is improved by the intraparticle diffusion related to the porous volume [48–50], which depends either on the pore size or on the type of particle, fully or superficially porous. For particles having a solid core, the porous volume is reduced, as well as the pore diffusion. Moreover, the narrow distribution of the

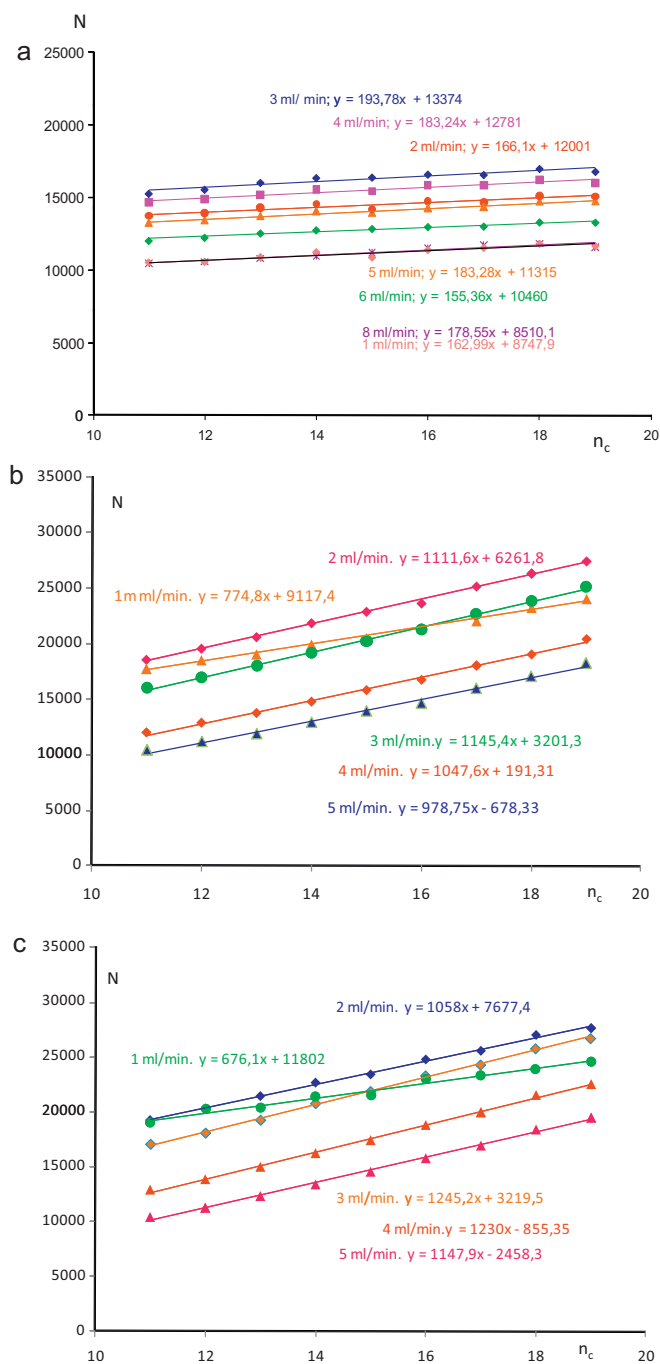


Fig. 6. Effect of the flow rate on the experimental plate number of alkylbenzenes ($n_c = 11–19$). (a) Luna C18(2) 5 microns 25 cm; (b) Kinetex C18; (c) Halo C18. Same mobile phase, outlet pressure and temperature as in Fig. 1.

superficially porous particles, leading to low eddy diffusion could be involved in such behavior. The reduced pore volume for HSS 5 micron phase, with a 15 cm column, could also explain the slightly positive slope observed for this column in Fig. 2.

Fig. 7 shows the N vs. n_c curves at different flow rates (varying from 1 to 5 mL/min) for a $\text{CO}_2/\text{acetonitrile}$ (90/10, v/v) mobile phase with the Kinetex C18 phase. The use of acetonitrile as modifier allows to reach lower H values (Fig. 5), as it does in RPLC with hydro-organic mobile phases when replacing methanol by acetonitrile, due to the greater diffusion coefficient of the compounds in less viscous fluids.

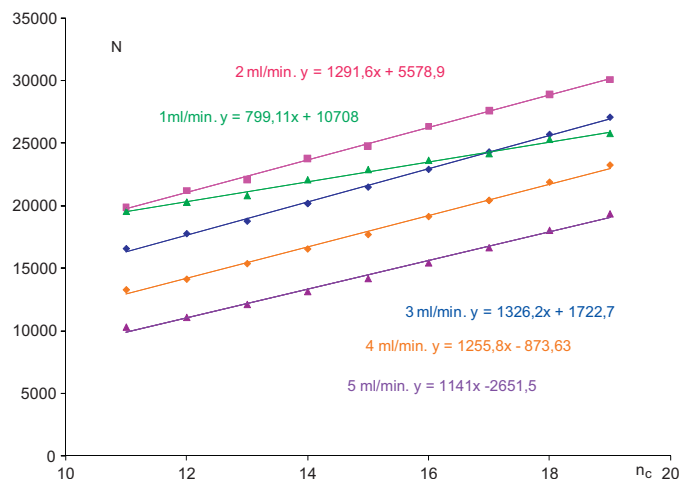


Fig. 7. Effect of the flow rate on the experimental plate number of alkylbenzenes ($n_c = 11–19$) on Kinetex C18. Mobile phase: $\text{CO}_2/\text{acetonitrile}$ 90/10 (v/v). Other conditions as Fig. 1.

At 25 °C and with 10% modifier, the replacement of methanol by acetonitrile induces only a slight pressure drop decrease, even at large flow rates, as seen in Fig. 1. The maximum theoretical plate number is equal to 30,000 for the C19 alkylbenzene at 2 ml/min, leading to an improved reduced h value of 1.92.

The same behavior is observed, i.e. the change in the slope of N vs n_c at 1 ml/min.

3.4. Temperature changes

Finally, the change in temperature was studied for Zorbax Eclipse Plus (25 cm; 5 microns) and for Kinetex C18 (15 cm; 2.6 μm), between 15 and 35 °C.

Fig. 8 shows the variation of N with n_c at different temperature values. Fig. 8a shows a classical kinetic behavior observed in SFC with insulated columns [45] for the Zorbax 5 microns phase. First, at 15 °C, and for all compounds, a lower efficiency is noticed due to the reduced diffusion of compounds into the mobile phase, such as it occurs in liquid, which probably reduces the speed of compounds exchange between the mobile and the stationary phases, mainly for the greatest alkylbenzenes having the lowest diffusion coefficient D_m .

The higher efficiencies are obtained from 20 to 30 °C, with no significant differences between the compounds whatever their retention factor. However, a decrease of efficiency is reported at 35 °C. This could be due to the radial negative temperature gradient (called negative in regards to the one reported in UHPLC), which is due to the fluid decompression along the column and the cooling effect related to this decompression [41–45]. For temperatures above 30 °C, the column walls could be at a significantly higher temperature than the center of the column. This induces radial viscosity, velocity and eluting power gradients.

Indeed, the lower temperature at the column center induces higher mobile phase viscosity, a higher eluting power, and a lower mobile phase velocity, in regards of these parameters near the wall of the column. This phenomenon was assessed elsewhere, both by temperature measurement and unexpected pressure drop variation in relation to temperature changes [41–45].

On the Kinetex column, Fig. 8b shows that 20 °C seems to be the optimal value with methanol/ CO_2 mobile phase, the theoretical plate number and the h value reach the high values obtained at 25 °C in acetonitrile/ CO_2 mixtures.

Because the pressure drop is higher with smaller particle sizes, the optimal temperature is slightly lower on Kinetex than on

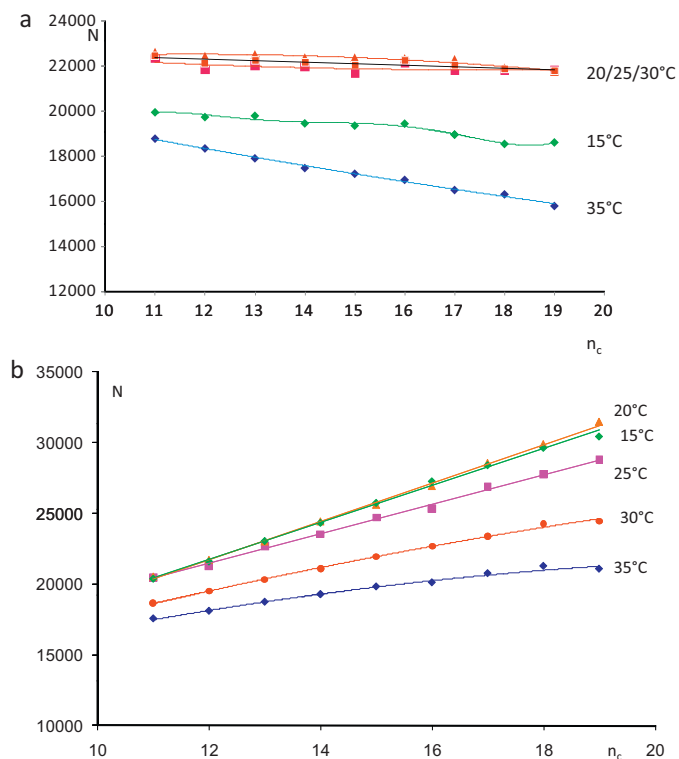


Fig. 8. Effect of temperature on theoretical plate number of alkylbenzenes ($n_c = 11-19$). Conditions as in Fig. 1, flow rate: 2 ml/min. (a) Zorbax Eclipse plus C18 (250 mm \times 4.6 mm; 5 microns); (b) Kinetex C18.

Zorbax. It could be explained by the greater decompression effect and the probably higher cooling effect on Kinetex (not measured). The values obtained at 15 °C are also quite good, showing now that the reduced diffusion of compounds into the mobile phase at lower temperature does not induce an efficiency decrease with superficially porous particles.

As reported for the column with fully porous particles, the increase in oven temperature from 25 to 35 °C decreases the

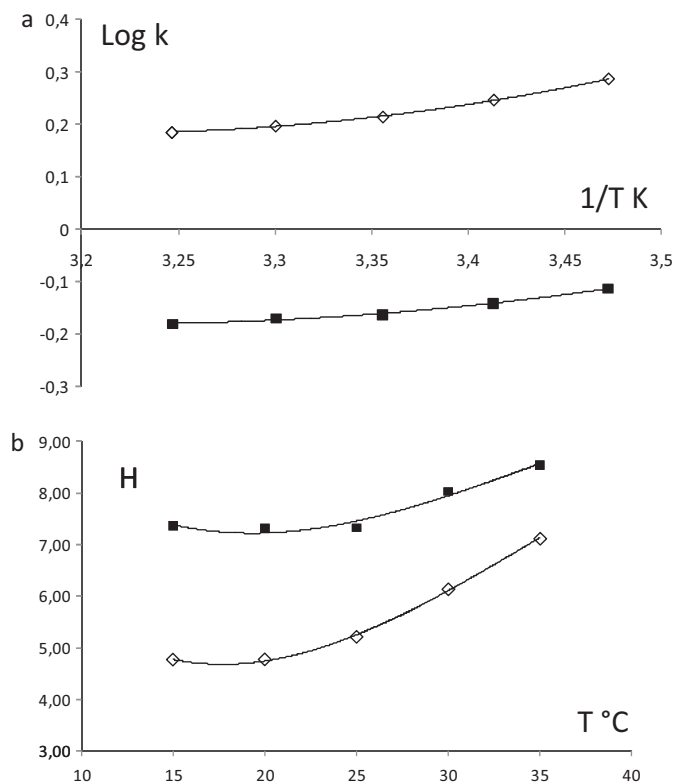


Fig. 9. Effect of temperature change from 15 to 35 °C on Kinetex C18. (a) On log k ; (b) on H . Open triangles: nonadecylbenzene; full squares: undecylbenzene.

theoretical plate number values, probably by favoring a negative radial temperature gradient. This decrease seems to be more significant for the most retained compounds, which have the greater residence time in the column, reinforcing the radial temperature hypothesis.

Fig. 9a and b displays the variation of log k vs. $1/T$ and H vs. T on the Kinetex C18 phase at 2 ml/min in CO₂/MeOH (90/10, v/v), for the two limit compounds (the least retained undecylbenzene and the most retained nonadecylbenzene). The increase in temperature

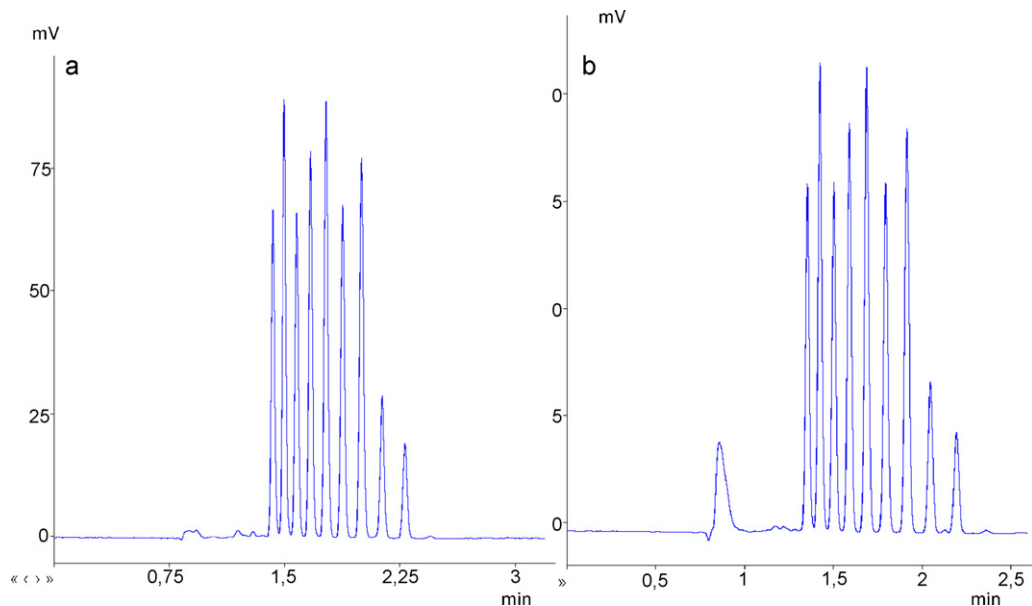


Fig. 10. Chromatograms of alkylbenzenes in SFC on Kinetex C18 column. Conditions: outlet pressure = 15 MPa; flow rate 2 ml/min. (a) CO₂/MeOH 90/10 (v/v); $T = 20$ °C; (b) CO₂/ACN 90/10 (v/v); $T = 25$ °C.

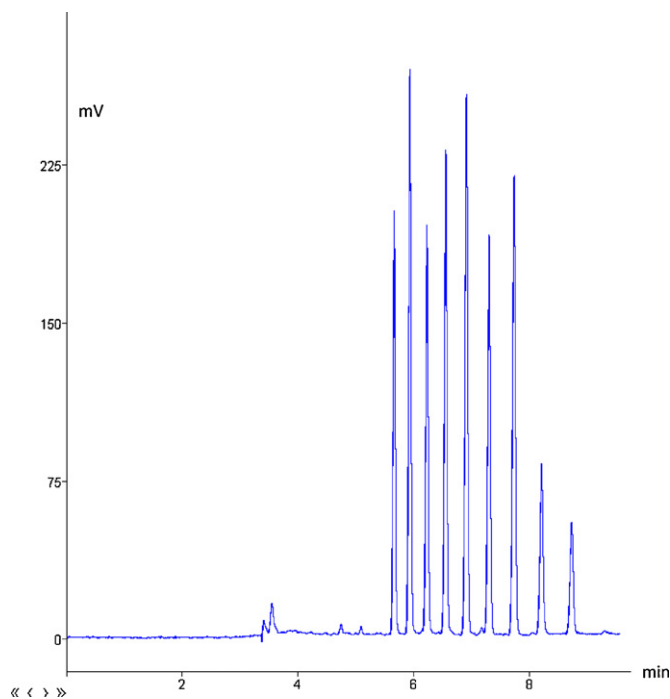


Fig. 11. Chromatogram of alkylbenzenes in SFC on four Kinetex C18 columns (total length 60 cm) with CO₂/MeOH mobile phase. Other conditions as Fig. 1.

favors the elution of the compounds (Fig. 9a), as it does in HPLC. This shows that the supercritical fluid behavior, in these conditions, is close to that of a liquid. In SFC, for lower amounts of methanol, or lower pressure drops, i.e. for mobile phases with lower density, the increase in temperature generally causes increased retention because of the density decrease of the mobile phase. This density decrease reduces the eluting power of the mobile phase, leading to increased retention factors [15,17].

Nevertheless, the retention variation is identical for the less and more retained compounds (the curves in Fig. 9a are parallel), whereas the H change is greater for the more retained compound (the slope of the lower curve in Fig. 9b is larger than that of the upper curve).

This differential H change could be due to the radial temperature effect, which decreases more the efficiency of the more retained compounds, i.e. for the compounds having the greater retention time.

To conclude on the great potential of the use of ODS superficially porous particles in SFC, Fig. 10 shows the separation of alkylbenzenes in MeOH/CO₂ and ACN/CO₂ mobile phases on the Kinetex C18 column. The separation is rather satisfactory, in a short retention time, less than 2.5 min. Due to the low fluid viscosity, column coupling is often performed in SFC to improve the theoretical plate number, i.e. the resolution [11,51,18,52,53]. Fig. 11 shows the same separation with four Kinetex columns (total length equal to 60 cm). The total analysis duration is around 9 min, and the theoretical plate number close to 120,000. No loss of efficiency was noticed due to the increase in the column length, due to tubing or density changes in the column entrance related to the high pressure drop. The peak capacity, calculated from the isocratic equation [54] is around 85.

This value is not very high, but when calculating the peak capacity per hour per MPa of pressure drop ($\Delta P = 23$ MPa, $P_{in} = 35$ MPa; $P_{out} = 12$ MPa), a value of 25 is reached, which is higher than the maximum value, equal to 18, reported for the separation of peptides with 60 cm of 5 microns pellicular Poroshell in HPLC, using an elution gradient and an optimistic peak width [9]. With an average peak width value, this value in HPLC would be lower than 10.

4. Conclusion

The use of ODS superficially porous particles in SFC allows the achievement of highly efficient separation, with or without column coupling. The high permeability of these particles favors the increase of the column length, i.e. the increase in resolution. Besides, despite this allowed column length increase which increases the retention time, the high elutropic strengths of CO₂/modifier phases avoid prohibitive analysis durations.

However, for superficially porous particles, surprising kinetic behaviors are reported in isocratic mobile phase conditions, which favor the efficiency of the most retained compounds, as a gradient elution usually does. These behaviors seem to depend on the residence time of the compounds in the column, i.e. in the flow rate of the mobile phase, and could be explained by trans column dispersion through the radial concentration gradient, which is better relaxed for fully porous particles, as it is for unblocked pores with fully porous ones [32]. This behavior also appears for 2.5 μ m fully porous particles, but to a lower extent, showing that not only the particle type but also the particle sizes are involved in the observed phenomenon.

As described for fully porous particles of 5 microns, the use of superficially porous particles at higher temperatures than the ambient one also leads to reduce theoretical plate numbers. This effect could be related to a radial temperature gradient due to the cooling effect of the fluid depressurization along the column, which depends on the pressure drop, and which could be higher with superficially porous bonded particles. Despite the high pressure drop reached with 4 coupled 15 cm columns, this effect seems to be avoided at 25 °C. However, no loss in efficiency was reported with superficially porous silica particles at 50 °C [23], showing potential differences in the observed behaviors related to the bonding of the particles.

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