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Comparison of the fast gradient performance of new prototype silica monolithic columns and columns packed with fully porous and core–shell particles

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ABSTRACT

The gradient elution performance of narrow-bore 2.3 mm × 50 mm (N733) and wider bore 3.2 mm × 50 mm (N648 and N655) prototype silica monolithic columns was investigated and compared to the performance of commercially available columns packed with sub-2 μm fully porous particles (2.1 mm × 50 mm, 1.7 μm BEH-C_{18}, Waters) and sub-3 μm superficially porous particles (2.1 mm × 50 mm, 2.7 μm Halo-ES-Peptide-C_{18} (AMT), 1.7 and 2.6 μm Kinetex-C_{18}, Phenomenex). Results show that the two wide monolithic columns show peak capacities similar to the one measured for the Kinetex column. In contrast, the narrow-bore monolithic column delivers a lower performance (−30%) than the BEH, the Halo and the Kinetex columns. This work stresses out the importance of reducing the extra-column band broadening contribution of HPLC instruments when short 2.1 mm I.D. columns are used. The part of the instrument contribution originating downstream the column is important for all compounds; the one originating upstream the column is significant only for weakly retained compounds.

1. Introduction

The pharmaceutical and food industries are constantly challenging column manufacturers to prepare more permeable columns providing smaller height equivalent to a theoretical plate (HETP) [1]. For a given maximum column inlet pressure, more permeable columns are always preferred because they allow shorter analysis times and a significant gain in time. High column efficiencies are required to provide sufficiently high resolutions of the sample components, meaning that long columns are usually needed. This classical chromatographic *conandrum* between fast and high-resolution separations has successfully brought column technology toward the preparation and packing of smaller particles (down to 1.7 μm) along with the development of very high pressure liquid chromatographs [2]. Fast and efficient analyses could then be achieved with small volume 2.1 mm × 50 mm columns but at pressures routinely exceeding 400 bar [3]. In order to help coping with the problem of very high pressures, sub-3 μm shell particles emerged in 2006 [4–6]. Columns packed with these materials offer optimum plate heights comparable to those measured with columns packed with sub-2 μm fully porous particles but have twice to thrice larger column permeabilities [7–10], allowing their use with standard 400 bar instruments, provided that the contribution of the chromatograph to band broadening be minimized by replacing the standard parts with less voluminous ones [11].

The first generation of silica monolithic columns was introduced twelve years ago. These columns provided an exceptionally high permeability, close to that of columns packed with 10 μm particles [12]. Nevertheless, their minimum plate heights was also high, around 20 μm, so that 10 cm long columns could barely provide 5000 plates. In contrast, 5 cm long columns (2.1 and 3.0 mm I.D.) packed with sub-2 μm fully porous particles [13] and 10 cm long columns (4.6 mm I.D.) packed with sub-3 μm shell particles [7] are now able to produce about 15 000 and 30 000 plates, respectively. This difference in performance explains the limited success of the monolithic columns of the first generation. Their decline was soon attributed to large trans-column velocity biases [14], a direct consequence of their preparation process: the production of the rods involves an exothermic sol–gel reaction and is followed by a drying step [15] causing a shrinking of the rod and a large external porosity (70%). This velocity bias was confirmed by local electrochemical detection of the eluting band of a non-retained species at different radial positions across the outlet column diameter [16,17]. Relative velocity biases of about 3% and 5% were measured for 4.6 and 10 mm I.D. silica rods, respectively. Recently, manufacturers prepared more radially homogeneous silica structures [18]. Merck Millipore (Darmstadt, Germany) released last October a second generation of 4.6 mm × 100 mm silica monolithic columns. Recent investigations of their performance showed that these columns

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Nomenclature

Roman letters

F_v	flow rate (m^3/s)
F_v	column phase ratio
G	intrinsic gradient steepness ($=S\Delta\varphi(t_0/t_G)$)
G	intrinsic gradient steepness of the last eluted compound ($=S_{last}\Delta\varphi(t_0/t_G)$)
G_{12}^2	gradient band compression factor
H	total column HETP (m)
$\overline{H}$	average column HETP experienced by the sample during gradient elution (m)
k	retention factor
k_i	retention factor of compound *i* in the RPLC checkout sample
k_0	retention factor extrapolated in pure water
K_0	specific permeability (m^2)
K_{Cell}	dispersion constant in Eq. (18)
K_{Inj}	dispersion constant in Eq. (21)
$k_{0,last}$	retention factor of the last retained compound extrapolated in pure water
k_F	apparent retention factor of the last eluted compound in gradient conditions
k_I	retention factor of the eluted compound when it enters the column
k_L	retention factor of the eluted compound when it exits the column
k_{last}	retention factor of the last eluted compound at the beginning of the gradient
l	capillary length (m)
L	column length (m)
P_c	theoretical peak capacity
$P_{c,exp}$	experimental peak capacity
r_c	capillary inner radius (m)
t	time variable (s)
t_D	dwell time (s)
t_0	column hold-up time (s)
t_9	gradient elution time of the last eluted compound of the RPLC checkout sample (s)
t_{last}	elution time of the last eluted compound in gradient conditions (s)
t_G	gradient time (s)
S	negative of the slope of the LSSM plot
S_{last}	LSSM slope parameter of the last eluted compound in gradient conditions
u_0	chromatographic linear velocity (m/s)
v_{Cell}	detection cell volume (m^3)
v_{Valve}	injection valve volume (m^3)
z	axial column coordinate (m)
$z_{catch,i}$	axial column coordinate at which the gradient is catching up with the compound *i* in the RPLC checkout sample (m)

Greek letters

ε_t	total porosity
ε_e	external porosity
ε_p	internal porosity
φ	volume fraction of acetonitrile in the mobile phase
φ_{start}	volume fraction of acetonitrile at the beginning of the gradient
φ_{end}	volume fraction of acetonitrile at the end of the gradient
φ_9	volume fraction of acetonitrile at which the last eluted compound of the RPLC checkout sample exits the column
$\Delta\varphi$	amplitude of the change in volume fraction during the gradient
Ψ	parameter defined in Eq. (7)
σ	peak standard deviation (s)
σ_t^2	total time variance (s^2)
$\sigma_{t,I}^2$	time variance associated with the band broadening taking place upstream the column (s^2)
$\sigma_{t,D}^2$	time variance associated with the band broadening taking place downstream the column (s^2)
$\sigma_{t,ex}^2$	extra-column time variance (s^2)
$\sigma_{t,Inj}^2$	time variance associated to the injected volume (s^2)
$\sigma_{t,seat}^2$	time variance associated with the band broadening taking place in the needle seat capillary (s^2)
$\sigma_{t,Viper}^2$	time variance associated with the band broadening taking place in the Viper connecting tube (s^2)
$\sigma_{t,Cell}^2$	time variance associated with the band broadening taking place in the detection cell (s^2)
$\sigma_{t,Connect}^2$	time variance associated with the band broadening taking place at the connections between the system parts (s^2)
ω	parameter defined in Eq. (6)
ω_9	baseline peak width of coumpound #9 in the RPLC checkout sample (s)
ρ	ratio of the solid core to the particle diameter for shell particles

have the efficiency of columns packed with 3.5 μm fully porous particles and the permeability of columns packed with 4.5 μm particles [19]. It was observed, however, that a small trans-column relative velocity bias subsists in these new columns (about to 1%). Kyoto Monotech (Kyoto, Japan) prepared prototype 3.2 mm × 50 mm silica monolithic columns that provide a performance equivalent to that of columns packed with 2 μm particles with the permeability of columns packed with 4 μm particles. Exceptionally, the minimum HETP of these latter columns was found to be smaller for non-retained analytes than for retained compounds in RPLC, demonstrating that this new silica structure combined with newly designed frits and endfittings generated monolithic columns having nearly no radial velocity biases.

The goal of this work is to assess the performance of the new prototype columns prepared by Kyoto Monotech (N731, N648, and N655) in gradient elution chromatography on a VHPLC instrument (1290 Infinity system from Agilent Technologies). Because the length of these columns is short (5 cm), they are well suited for fast analyses, if run at the maximum inlet pressure that these columns can withstand (~200 bar). Their peak capacity is compared to those of commercially available columns packed with sub-2 μm fully porous particles (1.7 μm BEH-C_{18}, Waters, Milford, USA) and sub-3 μm shell particles (1.7 and 2.6 μm Kinetex-C_{18}, Phenomenex, Torrance, USA) for the same analysis speed. Two test mixtures are used: the RPLC check out sample from Agilent Technologies (Little fall, DE, USA) and a home-made mixture of hydrocarbons produced by the green micro-algae *Botryococcus braunii* when incumbated under nitrogen stress conditions [20]. Theoretical expressions of the peak capacity (taking into account or not the peak compression during gradient elution) are used to extract an apparent isocratic HETP, *H*, assumed to be constant for all the components present in the mixture and for all mobile phase compositions. These *H* values permit a comparison of the relative performance of these new monolithic columns and of the best available particulate columns.