High-Speed HPLC Separations with Polar Fused-Core® Packings

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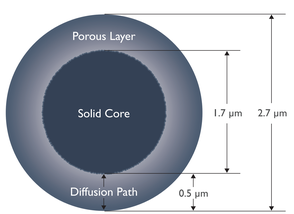
Abstract

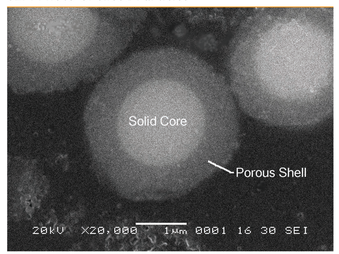
Separations by reversed-phase HPLC are most often performed using C18 bonded phase columns. The reasons for this popularity are 1) the C18 phase was the first reversed-phase packing in the commercial market, 2) the C18 phase has a reputation for being a very robust phase, and 3) there is a vast library of applications on the C18 phase on which to base starting conditions for new applications. Other reversed phase HPLC columns such as cyanopropyl, phenyl, amino, etc. are used significantly less often in published applications, but they do provide chromatographers with alternate selectivities for compounds relative to C18 phases. These other phases are used when C18 columns cannot separate compounds of interest or when orthogonal separations are required. This presentation introduces two new columns based on fused-core® particles that will provide alternate selectivities to the existing C8 and C18 fused-core columns. The advantages of the combination of the high-speed characteristics of the fused-core particles and the alternate selectivities of HILIC and embedded polar stationary phases will be demonstrated.

Fused-Core Particle Technology

Cartoon of Particle



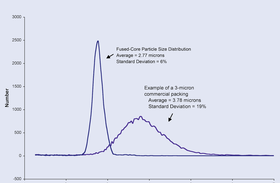
SEM Photo of Sliced Particles



Specifications for Fused-core Particles

- 2.7 µm overall diameter
- 1.7 µm solid core
- 0.5-µm-thick porous outer shell
- 9 nm pores in porous shell
- Highly purified Type B silica
- Surface area ~150 m2/g
- Pressures to at least 600 bar

Unusually Narrow Particle Size Distribution



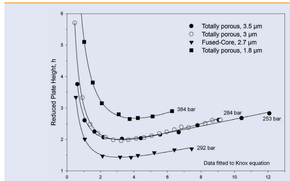
Theory

Van Deemter equation

$$H = A + B/u + Cu$$

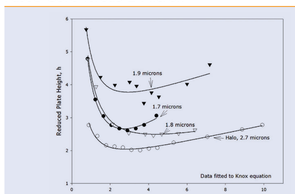
- A-term dominated by packed bed homogeneity
- C-term related to resistance to mass transfer in the porous structure
- Small A and C-terms preferred
- Short diffusion path due to thin shell results in smaller C-term
- High efficiencies (h<2!) due to uniform particle size leading to smaller A-term
- Moderate backpressure due to particle size being larger than sub-2-µm particles

van Deemter Plots of Totally Porous vs. Fused-Core Particles



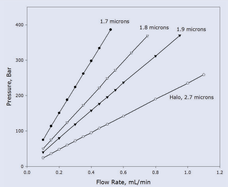
Columns 50 x 4.6mm; Mobile phase: 60% ACN/40% water
Bonded phase: C18; Temperature: 24 °C; Solute: naphthalene;

Fused-Core vs. Sub-2-Micron



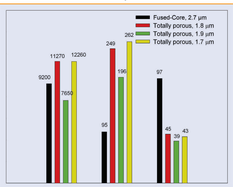
Columns: 2.1 x 50 mm
Solute: naphthalene

Back Pressure Plots for High-speed Columns



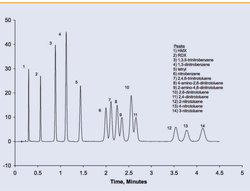
Columns: 50 x 2.1mm, C18; Mobile phase: 70% ACN/30% water
Temperature: 24°C; Agilent 1100

Small-particle Column Comparison



Columns: 2.1 x 50mm; Solute: acenaphthene, k=3.1 +/- 0.7
Mobile phase: 70% ACN/30% water; Temperature: 25 °C
All data taken at plate height minimum

Separation of Explosives

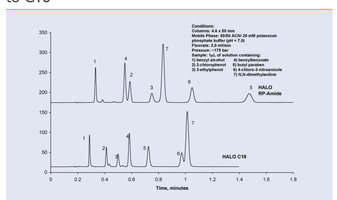


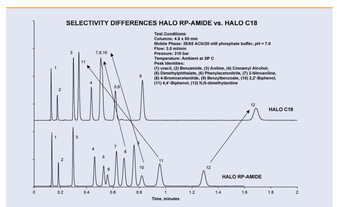
Column: 4.6 x 50 mm Halo C18; Mobile phase: 27% Methanol/73% Water;
Temperature: 40°C; Flowrate: 3.3 ml/min;
Detector: UV @ 254 nm; Pressure: 343 bar; Agilent 1100

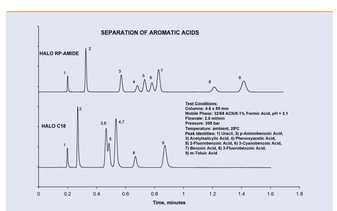
New Polar Phases Provide Alternate Selectivity to C18

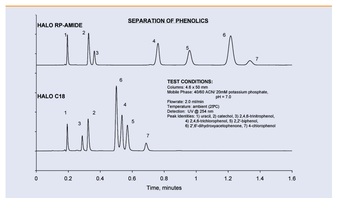
HALO RP-Amide (Polar-embedded Phase)
HALO HILIC (Bare Fused-Core Silica)

New RP-Amide Phase Provides Alternate Selectivity to C18

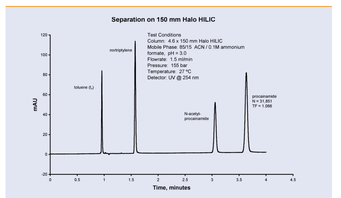


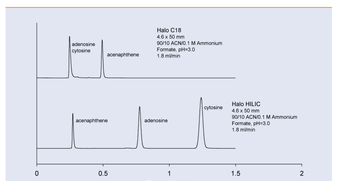


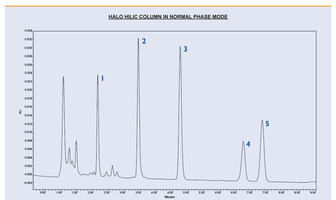




New HILIC Phase Provides Good Retention for Polar Solutes







Test Conditions:
Column: 4.6 x 150 mm HALO HILIC
Mobile Phase: 90.5/8.5/1 n-heptane/IPA/MeOH (0.1% water added)
Flowrate: 1.6 ml/min Temperature: Ambient (23.5°C)
Detection: UV at 260 nm
Peak Identities: 1) 25-hydroxy-VitD3,
2) 24(R),25-dihydroxy-VitD3,
3) 25(S),26-dihydroxy-VitD3,
4) 1,25-dihydroxy-VitD2
5) 1,25-dihydroxy-VitD3

Chromatogram courtesy Prof.Bouillon and Ivo Jans, LEGENDO, K.U.Leuven, Belgium

Conclusions

- Efficiency and separation speed rivals that of sub-2-micron columns, but with about one-half the pressure drop
- Produce unusual column efficiencies; reduced plate heights *h* of ~1.5 for small molecules
- Thin porous outer shell results in superior mass transfer kinetics and better efficiency at high mobile phase velocities, especially for larger molecules
- New polar phases offer alternatives for separation selectivity and easier method development