

Explaining the Small Particle Advantage

Faster Sample Throughput on a 1.9µm Pinnacle™ DB Column

by Rick Lake, Pharmaceutical Innovations Chemist

- Faster analyses, uncompromised chromatography using a 1.9µm Pinnacle™ DB small particle HPLC column.
- Narrow particle size distribution ensures consistent, high efficiencies and longer column lifetimes.
- 100% Restek manufactured—from base silica to final packed column—assures quality and reliability.



Restek is pleased to introduce an exciting new addition to our family of HPLC columns—the 1.9µm Pinnacle™ DB small particle column. Intended for use in ultra-high pressure liquid separations, the 1.9µm Pinnacle™ DB column combines the benefits of a novel technique with the unmatched quality you expect from Restek. From the manufacturing of the base silica through the packing of the column, Restek performs and tightly controls every step in the manufacturing process, guaranteeing ruggedness and reliability. Here we discuss how and why small particle HPLC columns work, and demonstrate the high efficiency, excellent peak symmetry, and fast analysis times that can be achieved on the 1.9µm Pinnacle™ DB column.

In HPLC column terminology, particle size refers to the mean diameter of the silica spheres used as the support material to which the stationary phase is bonded. Until recently, the practical particle size limit was around 3µm; smaller particle sizes resulted in backpressures above the limit of conventional LC systems. The advent of LC systems capable of handling higher backpressures (>10000psi) now allows chromatographers to realize the benefits of sub-2µm particle size columns. Smaller particles give rise to greater column efficiencies and a wider range of usable flow rates, resulting in better resolution and higher sensitivity with a significantly faster overall analysis time. **Figure 1** and **Table 1** illustrate the excellent peak shape and higher efficiency commonly seen when using a 1.9µm Pinnacle™ DB C18 column, compared to competitive columns.

To demonstrate the substantial gain in sample throughput that is possible on a small particle column, we assayed a series of parabens under identical conditions on both a C18 column with conventional dimensions and on a 1.9µm Pinnacle™ DB C18 column (**Figure 2**). When comparing the two columns, similar resolution was achieved in a much shorter analysis time on the 1.9µm Pinnacle™ DB C18 column. Next, we doubled the flow rate and assayed the same mix. The resolution and peak efficiencies again were comparable, but the analysis time was cut in half. This illustrates the considerable effect that small particles can have on chromatographic separations; a much wider range of usable flow rates translates into significantly faster analysis times—in this case 10-fold faster, with no loss in chromatographic quality.

The particle size of an HPLC column is actually the mean of the distribution of all particles used in the manufacturing of the column. In practice, the smaller the particle size distribution, the more uniformly packed the column will be, resulting in higher efficiencies. This distribution is even more critical when manufacturing columns with particle sizes less than 2µm. If the distribution contains many larger particles and is not tightly controlled, the efficiency of the column will suffer and column-to-column reproducibility will vary. More importantly, if the column contains particles less than 1µm (termed "fines"), clogging of the column frit and unwanted column backpressure can result. Restek's 1.9µm Pinnacle™ DB columns have narrow, symmetric particle size distributions, containing no particles less than 1µm in diameter. **Figure 3** illustrates this exceptional distribution, which is tighter and more accurate than competitive sub-2µm columns.

Restek's 1.9µm Pinnacle™ DB columns offer practical advantages for today's chemist across a wide range of analytes from acidic to basic. For higher sample throughput, matched with the reliability and ruggedness of a

RESTEK PRODUCTS

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Small Particle
HPLC Columns

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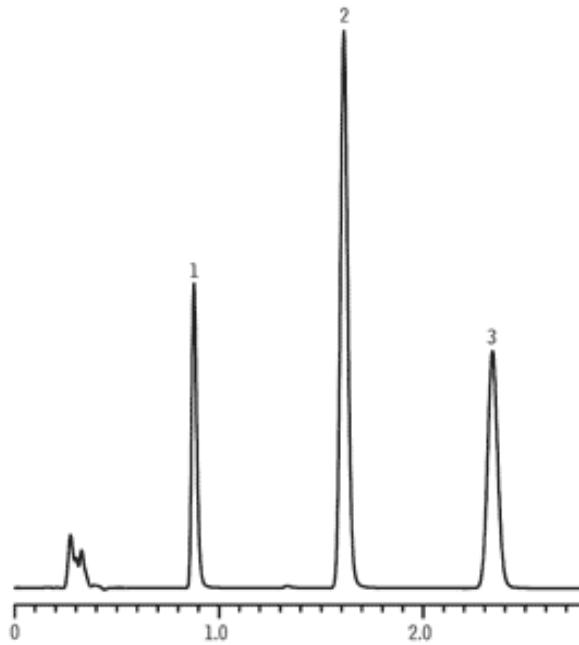
column made entirely by chromatographers for chromatographers, reach for Restek small particle HPLC columns.

Table 1 Restek's 1.9 μ m Pinnacle™ DB offers the highest efficiency of all columns tested (data from the biphenyl peak of a reversed phase test mix).

Column	Efficiency (n/m)	Pressure (psi)	Asymmetry
1.9 μ m Pinnacle™ DB	217,619	4,500	1.10
Competitor A	177,999	4,400	1.13
Competitor B	188,508	4,300	1.09

Figure 1 Excellent peak symmetry and efficiency on a 1.9 μ m Pinnacle™ DB C18 column, using a reversed phase test mix.

1. uracil 3.00mg/mL **Performance:** (calculations for biphenyl)
2. benzene 0.02mg/mL **Efficiency:** 217,619n/m
3. naphthalene 0.50mg/mL **Asymmetry:** 1.10
4. biphenyl 0.06mg/mL **Pressure:** 4,500psi



Sample:

Inj.: 2 μ L
Sample diluent: water:methanol (25:75)

Sample temp: ambient

Column: Pinnacle™ DB C18

Cat.#: 9414252

Dimensions: 50 x 2.1mm

Particle size: 1.9 μ m

Pore size: 140 \AA

Conditions:

Mobile phase: water:acetonitrile (45:55)

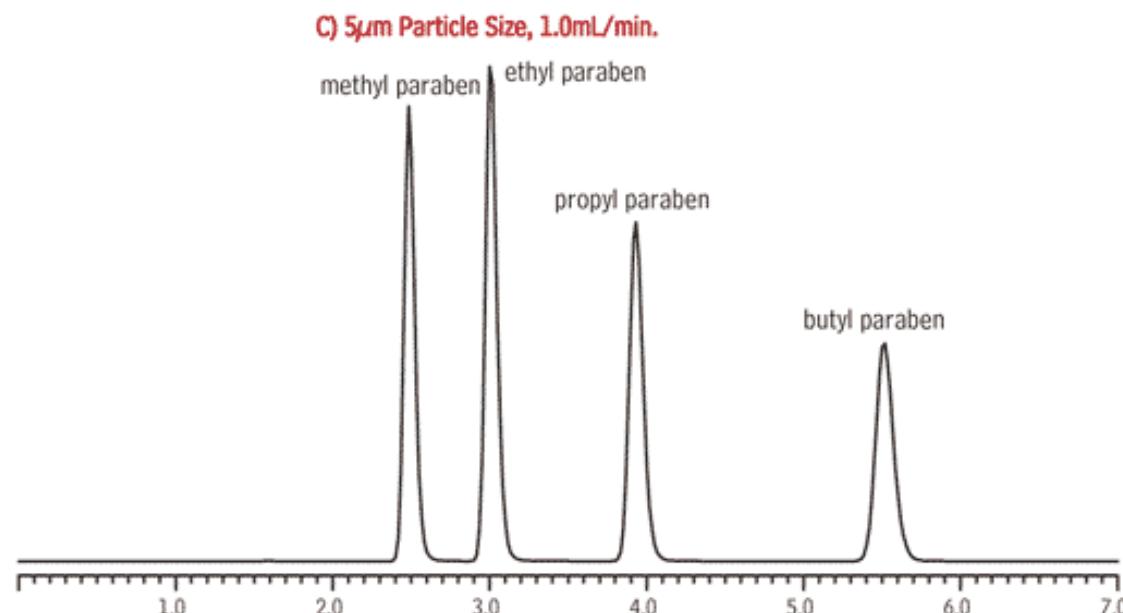
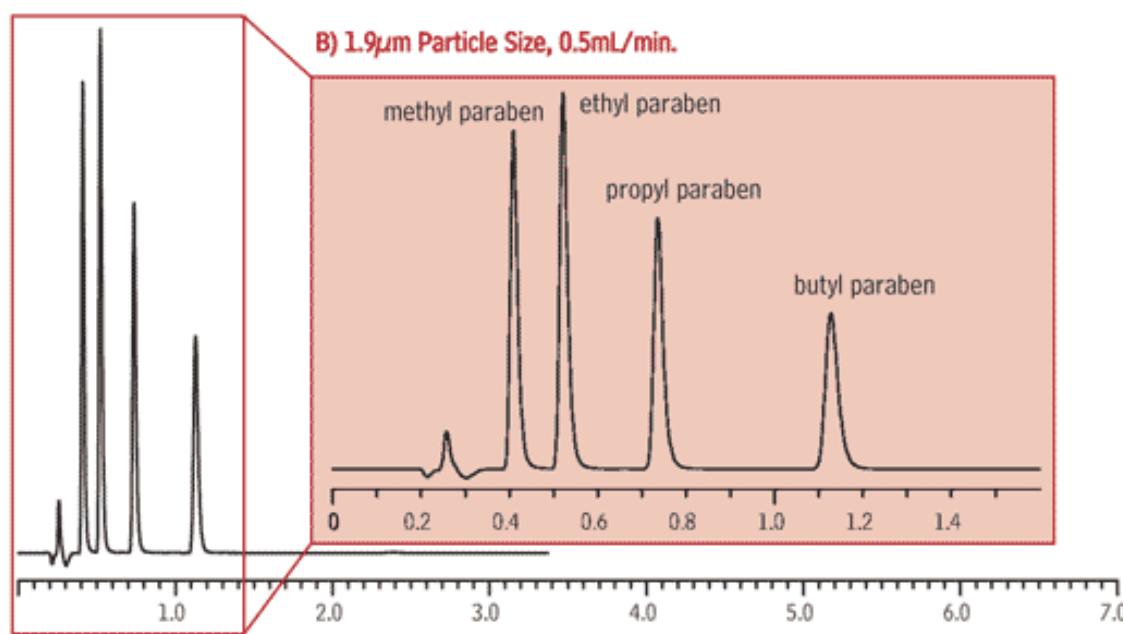
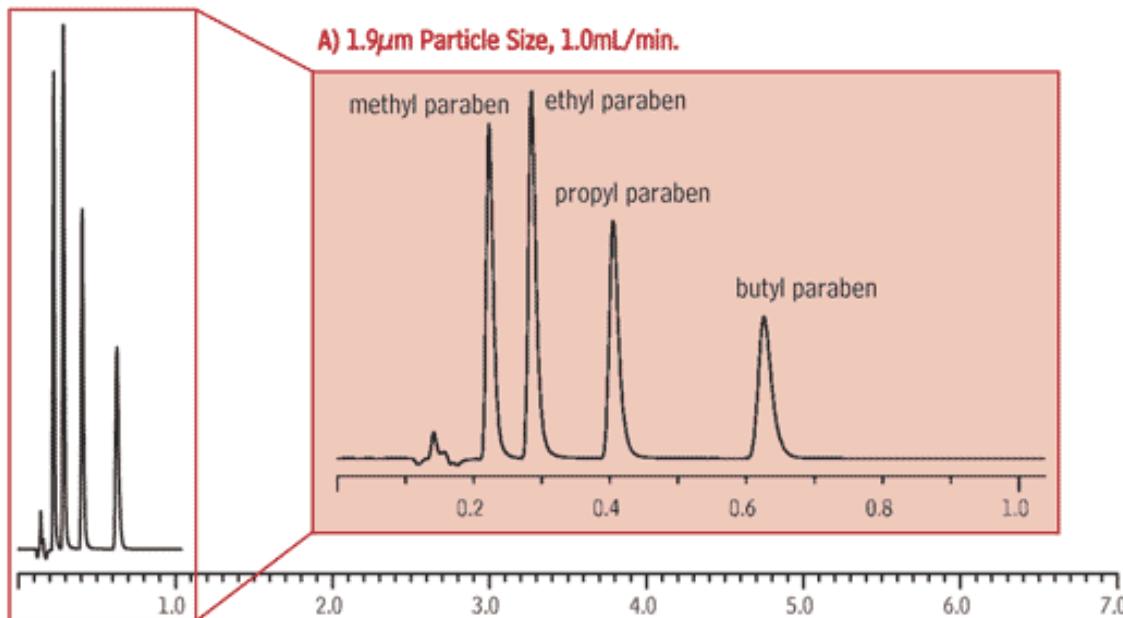
Flow: 0.4mL/min.

Temp.: 25°C (or ambient)

Det.: UV @ 254nm

LC_EX0427

Figure 2 Restek's 1.9 μ m Pinnacle™ DB columns can dramatically increase sample throughput—with no loss in resolution.



Column: A. Pinnacle™ DB C18, 1.9 μ m 50 x 2.1mm (cat.# 9414252)
 B. Pinnacle™ DB C18, 1.9 μ m 50 x 2.1mm (cat.# 9414252)
 C. Pinnacle™ DB C18, 5 μ m 150x 4.6mm (cat.# 9414565)

Inj.: A. 2 μ L (each compound 100 μ g/mL mobile phase)
 B. 2 μ L (each compound 100 μ g/mL mobile phase)
 C. 10 μ L (each compound 100 μ g/mL mobile phase)

Mobile phase: 50:50 0.1% acetic acid:acetonitrile

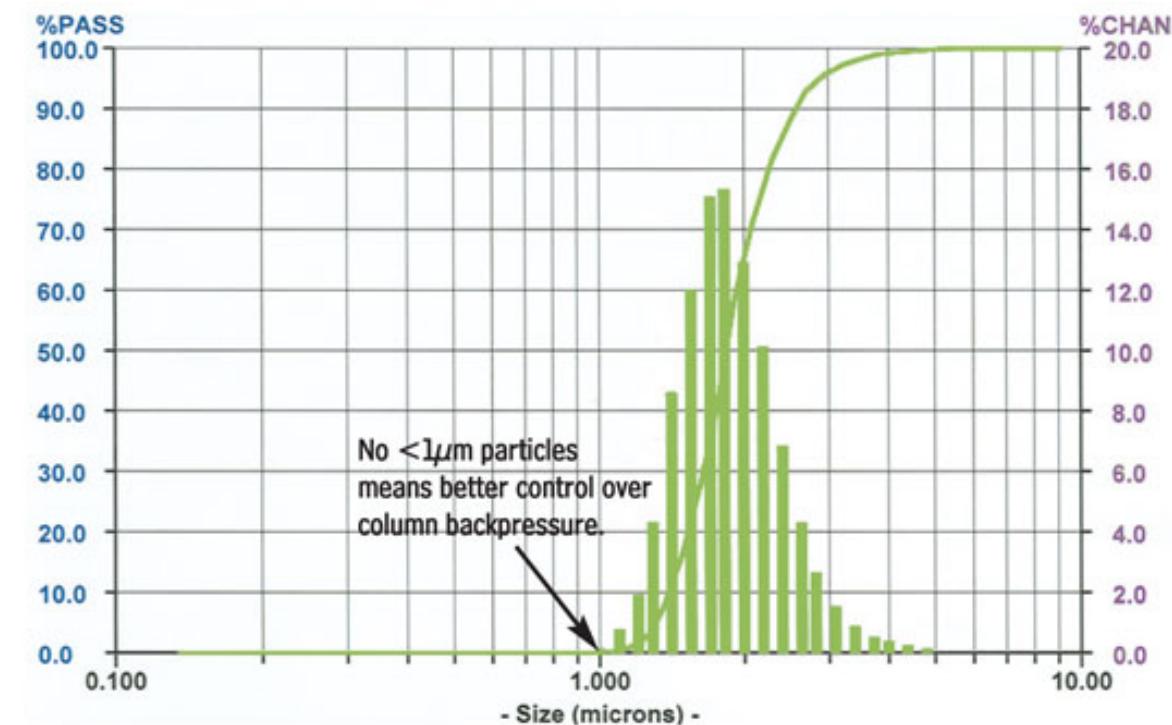
Flow: A. 1.0mL/min.
 B. 0.5mL/min.
 C. 1.0mL/min.

Temp.: ambient

Det.: UV @ 254nm

A. LC_PH0437
 B. LC_PH0436
 C. LC_PH0435

Figure 3 Pinnacle™ DB silica particle size distribution shows an exceptionally accurate mean and a tight, symmetrical distribution around 1.9 μ m; no <1 μ m particles are present, for protection against excessive backpressure.



	Target Particle Size (μ m)	Actual Mean Particle Size (μ m)	Standard Deviation	Particles Present <1 μ m
Column				
1.9 μ m Pinnacle™ DB	1.9	1.952	0.437	no
Competitor A	1.7	1.993	0.529	no
Competitor B	1.8	1.832	0.468	yes