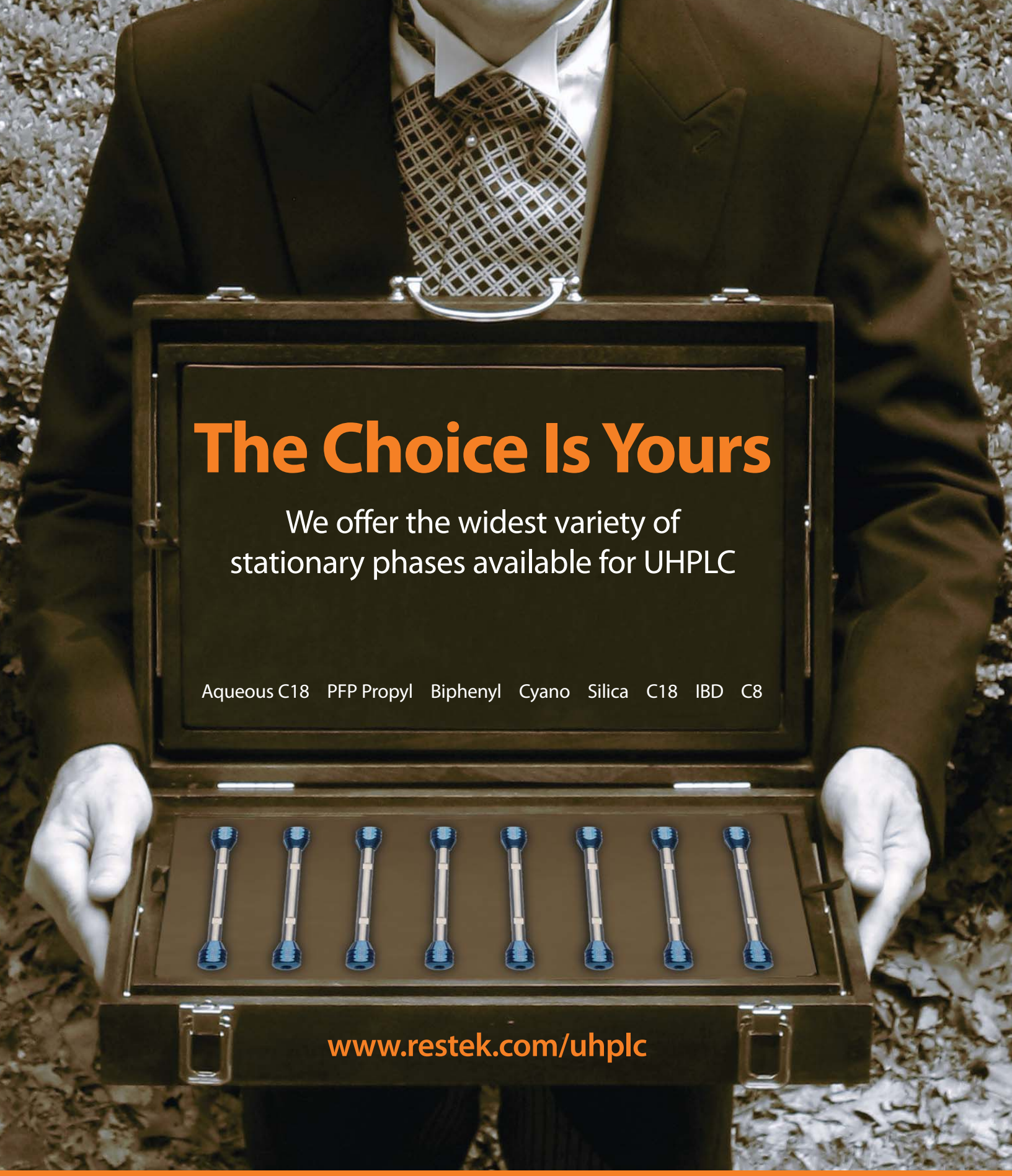



RESTEK

Chromatography Products

www.restek.com

# 1.9µm Pinnacle™ DB Columns Give You the Small Particle Advantage

## NEW! 8 Stationary Phases Now Available!

- Widest variety of stationary phases in a <2µm particle size.
- 100% Restek manufactured, from base silica to final packed column.
- Take full advantage of UHPLC: increased efficiency and higher sample throughput.

Restek continues to expand its offering of stationary phases on 1.9µm Pinnacle™ DB columns. Intended for use in ultra-high pressure liquid chromatography (UHPLC), each 1.9µm Pinnacle™ DB column combines the benefits of this popular technique with the unmatched quality that you expect from Restek HPLC. For even more method development options, we now offer 8 stationary phases, including the new Pinnacle™ DB Cyano, Pinnacle™ DB C8, and Pinnacle™ DB IBD (intrinsically base deactivated) phases.

### Faster Analyses, Uncompromised Chromatography

In UHPLC, smaller particle sizes provide greater column efficiencies and a wider range of usable flow rates, giving better resolution, higher sensitivities, and shorter analysis times. Figure 1 shows the higher efficiency and excellent peak shape characteristic of a 1.9µm Pinnacle™ DB C18 column.

Shorter analysis times are possible with smaller particle size packings for two reasons. First, the length of the column can be decreased as the particle size decreases and the efficiency increases, so equivalent resolution is possible with shortened analysis times. Second, higher flow rates can be used without a substantial loss in resolution. This is arguably the most significant advantage of <2µm particle sizes, since doubling the flow rate cuts the analysis time in half. The wider range of usable flow rates is possible due to flattening of the van Deemter curve as the particle size decreases, as shown in Figure 2.

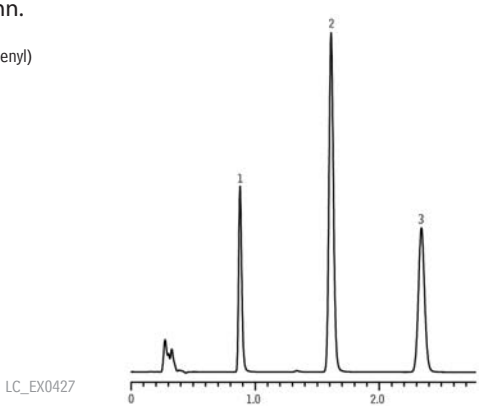
To demonstrate the run time advantage of a <2µm particle size, we assayed parabens under conditions resulting in comparable linear velocities on a 5µm C18 column and a 1.9µm Pinnacle™ DB C18 column (Figure 3 B & C). Similar resolution was observed with a much shorter analysis time on the 1.9µm Pinnacle™ DB C18 column. Further, we doubled the flow rate on the 1.9µm column and, while maintaining resolution and efficiency, the analysis time was reduced by another 50% (Figure 3 A). Smaller particle size packings allow a much wider range of usable flow rates, giving significantly faster analysis times with no loss in chromatographic quality.

**Figure 1** Excellent peak symmetry and efficiency using a 1.9µm Pinnacle™ DB C18 column.

Performance: (Calculations for biphenyl)
Efficiency: 217,619N/m
Asymmetry: 1.10
Pressure: 4,500psi

**Peak List:**

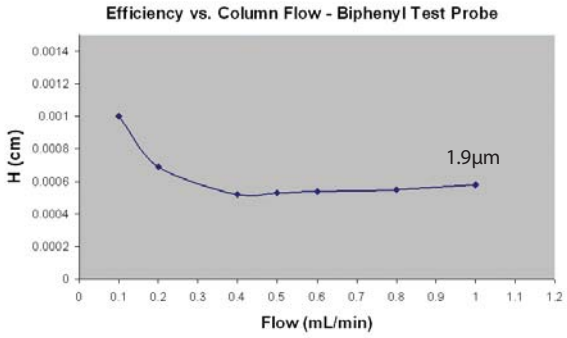
| | | |
|---|---|---|
| 1. | benzene | 0.02mg/mL |
| 2. | naphthalene | 0.50mg/mL |
| 3. | biphenyl | 0.06mg/mL |




Sample: Inj.: 2µL, HPLC Reversed Phase Test Mix #1 (cat.# 35005), Sample diluent: water:methanol (25:75), Sample temp.: ambient, Column: Pinnacle™ DB C18, Cat.#: 9414252, Dimensions: 50mm x 2.1mm, Particle size: 1.9µm, Pore size: 140Å, Mobile phase: water:acetonitrile (45:55), Flow: 0.4mL/min., Temp.: 25°C, Det.: UV @ 254nm

**Figure 2** The Van Deemter plots show that <2µm particle size columns offer faster analysis times by accommodating a wider range of usable flow rates.

(A) Pinnacle™ DB 1.9µm.




(B) 3, 5, & 10µm particle sizes.

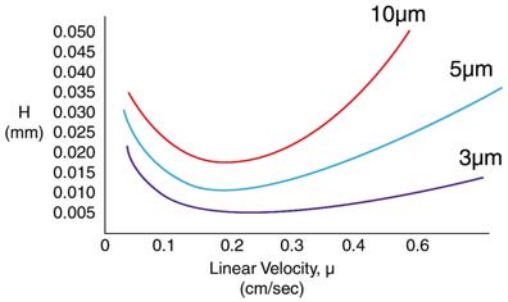
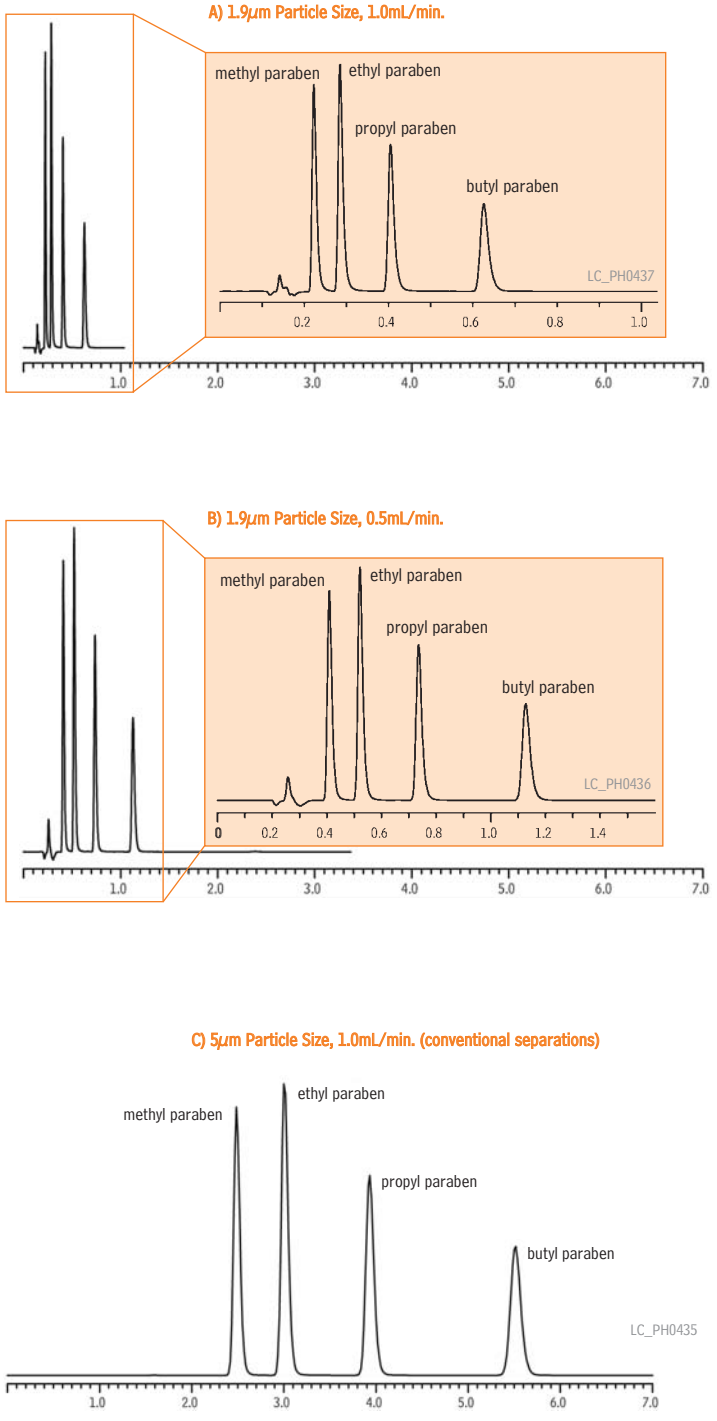


**Figure 3** 1.9µm Pinnacle™ DB columns offer a wider range of usable flow rates, dramatically increasing sample throughput—with no loss in resolution.

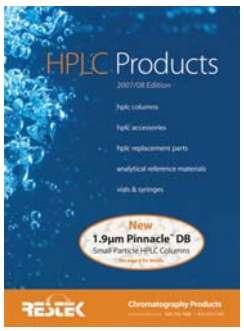



All chromatograms; Column: Pinnacle™ DB C18, Pore size: 140Å; Sample Conc.: ~100µg/mL each component in mobile phase (50:50 0.1% acetic acid:acetonitrile), Temp.: Ambient, Det.: UV @ 254nm

A) Inj.: 2µL, Cat.#: 9414252; Dimensions: 50mm x 2.1mm; Particle size: 1.9µm
B) Inj.: 2µL, Cat.#: 9414252; Dimensions: 50mm x 2.1mm; Particle size: 1.9µm
C) Inj.: 10µL, Cat.#: 9414565; Dimensions: 150mm x 4.6mm; Particle size: 5µm

## Narrow Particle Size Distribution—High Efficiencies and Longer Column Lifetimes

The specified particle size for packing material in an HPLC column is actually the mean of the distribution of all particles used. In theory, the tighter the particle size distribution, the more uniformly packed the columns will be, resulting in higher efficiencies (i.e. more theoretical plates). Particle size distribution is even more critical when manufacturing columns with particle sizes <2µm. If the packing contains many larger particles and the distribution is not reproducible from lot to lot, the efficiency of the column will decrease and column-to-column reproducibility will suffer. More importantly, if the column contains particles with a diameter less than 1µm (termed "fines"), clogging of the column frit and excessively high column backpressures can result. Restek 1.9µm Pinnacle™ DB columns have a narrow, reproducible particle size distribution and contain no particles <1µm in diameter (Figure 4, next page).




***HPLC Columns and Accessories***
(lit. cat.# 58059241C)

In addition to general-purpose and special-purpose HPLC columns and guard columns, this 100-page catalog contains instrument parts, innovative tools, and other accessories, and many example chromatograms from analyses on Restek columns. Columns are prepared from seven distinct lines of silica with characteristics tailored to meet specific analytical requirements. New products include 1.9 µm small particle columns, pHidelity™ pH stable columns, and capillary LC columns.




***Optimize Selectivity & Efficiency in UHPLC Separations With More Stationary Phase Choices on 1.9µm Pinnacle™ DB HPLC Columns***
(lit. cat.# 580202)

Ultra-high pressure liquid chromatography (UHPLC) can significantly increase efficiency and produce faster separations. The small particle sizes used in UHPLC improve efficiency; however, selectivity is still the most important factor affecting compound resolution. Here we demonstrate the importance of stationary phase choice in UHPLC separations. By optimizing selectivity for your analytes of interest, faster separations can be achieved without compromising resolution.

## Importance of Selectivity in UHPLC

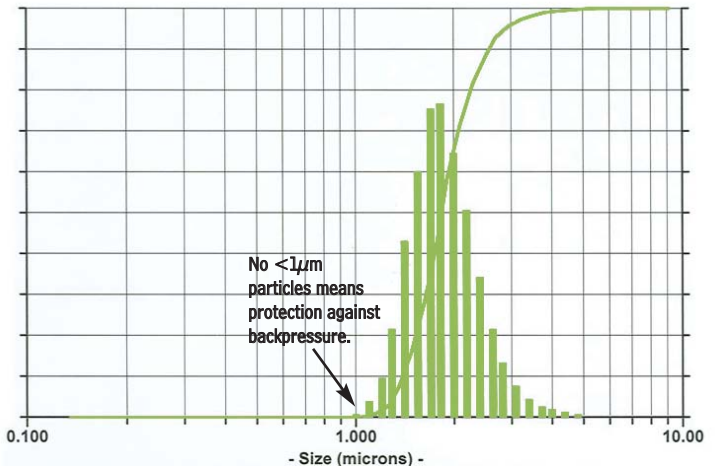
While efficiency can be increased and analysis times can be decreased using a <2µm particle size, selectivity remains the most important tool for optimizing overall chromatographic resolution. A stationary phase that produces optimum selectivity for the specific compounds of interest means the full benefits of UHPLC can be realized. For this reason, Restek has developed a complete line of stationary phase chemistries on our 1.9µm Pinnacle™ DB columns. Our current offering includes eight different phases: C18, Aqueous C18, Biphenyl, PFP Propyl (pentafluorophenyl propyl), Silica, Cyano, IBD (intrinsically base-deactivated), and C8. We also offer these same chemistries on the same Pinnacle™ DB silica in 3µm and 5µm formats, for easy method transfer between HPLC and UHPLC. Other particle sizes, suitable for preparative LC, are available upon request. Figure 5 shows the wide range of chemistries available for 1.9µm Pinnacle™ DB columns.

## Enhancing Selectivity through Unique Stationary Phases: Pinnacle™ DB Biphenyl and Pinnacle™ DB PFP Propyl

The two phenyl rings of the Pinnacle™ DB Biphenyl stationary phase interact with unsaturated and aromatic compounds through π-π interactions (Figure 5). When comparing biphenyl and conventional C18 phases, the Pinnacle™ DB Biphenyl column provides a distinct selectivity advantage for compounds that differ by level and position of unsaturation, such as in a series of closely-related steroidal compounds. When using the enhanced selectivity of the Pinnacle™ DB Biphenyl stationary phase in a highly efficient 1.9µm particle size column, fast, highly selective separations are possible. A 1.9µm Pinnacle™ DB Biphenyl column can separate the 7 hormones shown in Figure 6 in less than 2 minutes, a feat not possible on a conventional C18 phase.

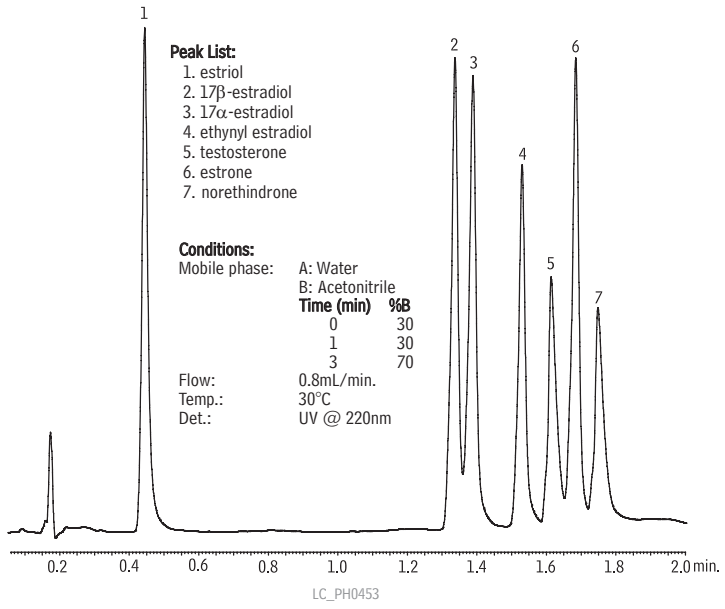
The Pinnacle™ DB PFP Propyl is extremely retentive and selective for organohalogens or other compounds containing basic or electronegative functionalities. In the analysis of halogenated drug compounds, such as benzodiazepines, the enhanced selectivity of the PFP Propyl phase is a significant advantage over conventional alkyl phases. In an assay of 8 benzodiazepines and 2 metabolites, complete resolution was possible in just over 4 minutes, as shown in Figure 7 (page 6). To get acceptable resolution with a C18 phase, a significantly slower mobile phase gradient (and longer analysis time) is necessary. The Pinnacle™ DB PFP Propyl column better retains the more polar metabolites, allowing for a simple mobile phase gradient and high sample throughput.

**Figure 4** Pinnacle™ DB silica particle size distribution shows a tight, symmetrical distribution around 1.9µm, with no <1µm particles.



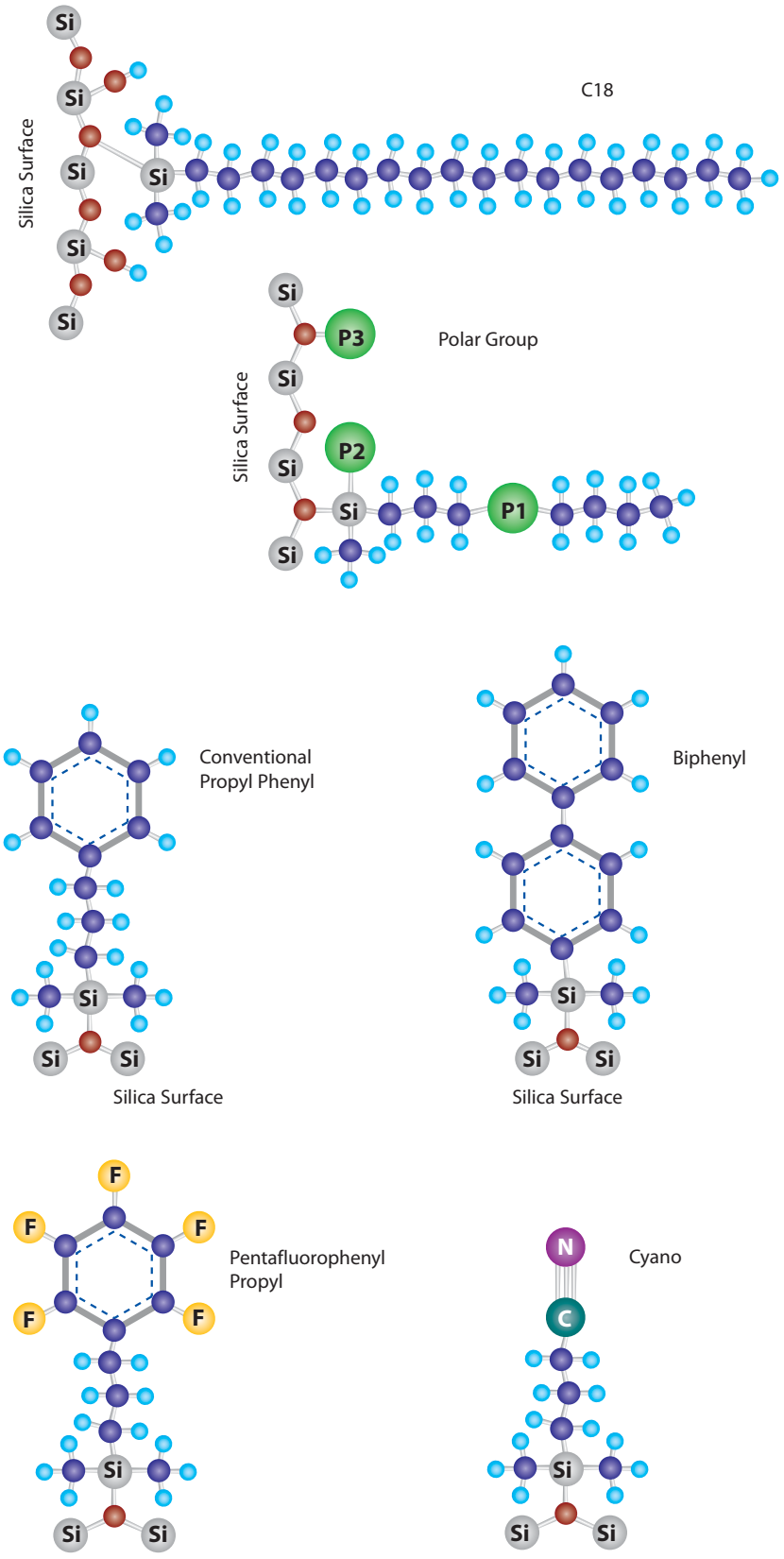

| Column | Listed Particle Size (µm) | Actual Mean Particle Size (µm) | Standard Deviation | Particles present <1µm |
|---|---|---|---|---|
| 1.9 Pinnacle™ DB | 1.9 | 1.952 | 0.437 | No |
| Manufacturer A | 1.7 | 1.993 | 0.529 | No |
| Manufacturer B | 1.8 | 1.832 | 0.468 | Yes |

**Figure 6** Restek's 1.9 µm Pinnacle™ DB Biphenyl columns are highly selective for steroids, making an extremely fast and selective analysis.




**Sample:** Inj.: 1µL; Conc.: 100µg/mL each component; Sample diluent: water:methanol (50:50)
**Column:** **1.9µm Pinnacle™ DB Biphenyl;** Cat.# 9409252; Dimensions: 50mm x 2.1mm; Particle size: 1.9µm; Pore size: 140Å
**Instrument:** Jasco X-LC

**Figure 5** Stationary phase chemistries available.




### Alkyl phases (e.g. C18)

Pinnacle™ DB C18
Pinnacle™ DB C8
Pinnacle™ DB Aqueous C18

Alkyl-based stationary phases, such as C18, are best suited for analyzing hydrophobic molecules with a high carbon:heteroatom ratio.

### Alkyl phase with polar functional group

Pinnacle™ DB IBD

An alkyl-based stationary phase with either an embedded polar group (P1), a polar side chain (P2), or a polar end-cap (P3), has significantly greater interaction with polar compounds than a traditional alkyl phase.

### Phenyl & Biphenyl phases

Pinnacle™ DB Biphenyl

Phenyl stationary phases interact with compounds containing aromatic groups or unsaturated bonds through π-π interactions. The biphenyl stationary phase has even greater interaction due to the higher concentration of aromatic rings.

### Cyano & Fluorinated phases

Pinnacle™ DB PFP Propyl
Pinnacle™ DB Cyano

Fluorinated phases (such as the pentafluorophenyl propyl (PFP Propyl)) and cyano-based phases interact strongly with basic, nitrogen-containing and halogenated analytes.

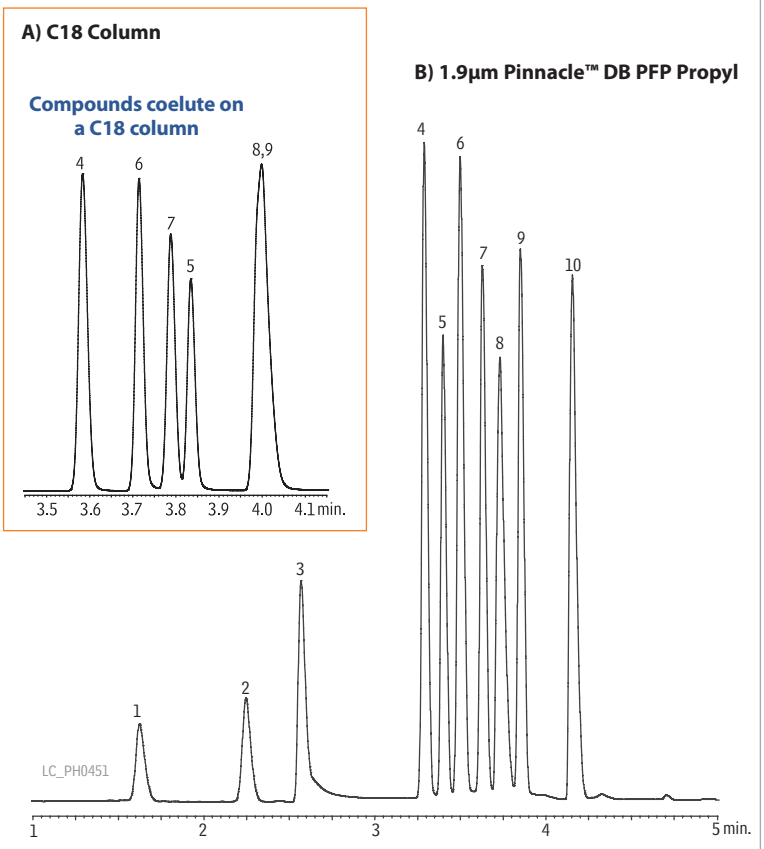
## Easily Transfer Methods to UHPLC Using Fully Scalable Pinnacle™ DB Columns

UHPLC is commonly used in two ways. First, to increase sample throughput, a conventional HPLC analysis can be scaled down to UHPLC. Alternatively, to lessen time in method development, a preliminary separation can be developed in UHPLC and then scaled up to conventional HPLC for routine analysis. In both cases, using comparable column chemistries and fully scalable columns, like the Pinnacle™ DB column line, will ease method transfer by maintain existing selectivity. For the analytical column to maintain selectivity, the pore size, carbon load, and support material must remain the same. In addition, when scaling an analysis and decreasing particle size and column dimensions, it is equally important that certain operating conditions are adjusted properly.

An analysis of sulfonamides can illustrate how easily Pinnacle™ DB columns can be scaled down from HPLC to UHPLC. Sulfonamides are commonly assayed on alkyl phases; however, their fused ring structure (Figure 8) shows enhanced selectivity when using a biphenyl phase that is capable of π-π interactions. From the initial operating conditions, some simple calculations can be performed to find the comparable analytical parameters appropriate for maintaining the existing chromatography, while reducing the analysis time. During a scaling procedure, column length, flow rate, injection volume and the time program for gradient analyses should be adjusted. When using these calculations to scale down from a 5µm particle size column (150mm x 4.6mm) to a 1.9µm particle size column (50mm x 2.1mm), we can see that the separation of sulfonamides is maintained, while the analysis time is greatly reduced (Figure 9). (For the complete article, including equations, please refer to www.restek.com/aoi_pharma_A020.asp.) Using fully scalable Pinnacle™ DB columns for your analytical methods, both HPLC and UHPLC, makes method transfer easy and reliable.

Restek 1.9µm Pinnacle™ DB columns, in a full range of stationary phase chemistries, allow you to fully benefit from a smaller particle size. For optimized separations and higher sample throughput, reach for Restek UHPLC columns & get the small particle advantage!

**Figure 7** Fast, selective analysis of benzodiazepines is made possible by combining the speed of UHPLC with the enhanced selectivity of the 1.9µm Pinnacle™ DB PFP Propyl column.




| Peak List: | Conc (µg/ml) |
|---|---|
| 1. 7-amino clonazepam* | 20 |
| 2. 7-amino flunitrazepam* | 20 |
| 3. bromazepam | 100 |
| 4. oxazepam | 100 |
| 5. lorazepam | 100 |
| 6. clonazepam | 100 |
| 7. nitrazepam | 100 |
| 8. nordiazepam | 100 |
| 9. flunitrazepam | 100 |
| 10. diazepam | 100 |

* metabolite

**Sample:**
Inj.: 1µL
Conc.: as listed
Sample diluent: starting mobile phase (80:20 A:B)

**Column:** **A. C18 Column**
**B. 1.9µm Pinnacle™ DB PFP Propyl**
Cat.#: A. 9414212
B. 9419212
Dimensions: 100mm x 2.1mm
Particle size: 1.9µm
Pore size: 140Å

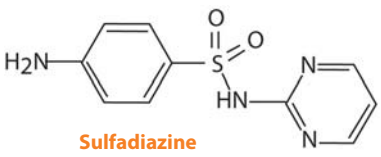
**Instrument:** Jasco X-LC

**Conditions:**
Mobile phase: A: 0.1% formic acid in water
B: 0.1% formic acid in acetonitrile

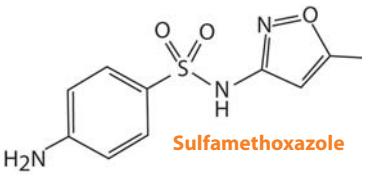
| Time (min.) | %B |
|---|---|
| 0 | 20 |
| 1 | 20 |
| 6 | 80 |

Flow: 0.6mL/min.
Temp.: 40°C
Det.: UV @ 254nm

**Figure 8** Chemical structures for example sulfonamides.

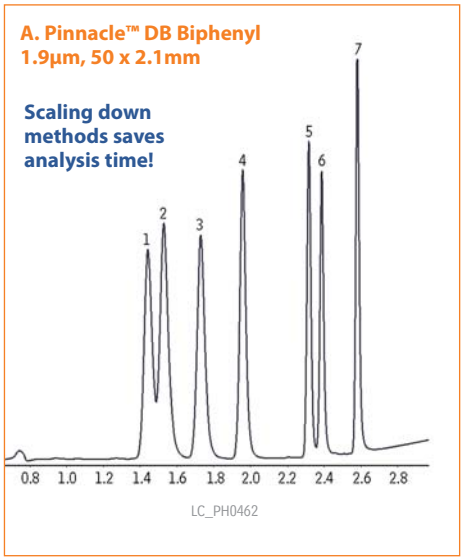


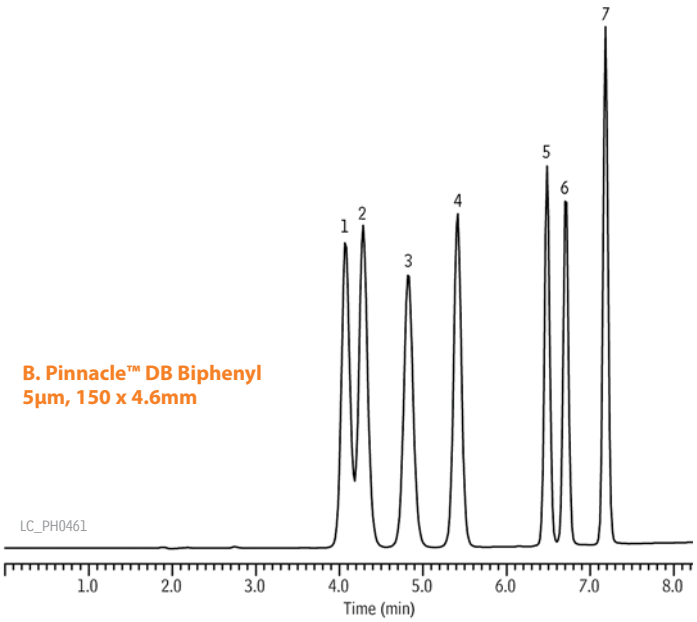

Sulfadiazine




Sulfamethoxazole

**Figure 9** Restek's Pinnacle™ DB 1.9µm columns can easily be scaled from HPLC to UHPLC and vice versa.




**Peak List:**
1. sulfadiazine
2. sulfathiazole
3. sulfamerazine
4. sulfamethazine
5. sulfachlorpyridazine
6. sulfamethoxazole
7. sulfamethoxine

**Sample:**
Inj.: 10µL
Conc.: 100µg/mL
Sample diluent: starting mobile phase (80:20 A:B)

**Column:** **A. 1.9µm Pinnacle™ DB Biphenyl**
Cat.#: 9409252
Dimensions: 50mm x 2.1mm
Particle size: 1.9µm

**Column:** **B. Pinnacle™ DB Biphenyl**
Cat.#: 9409565
Dimensions: 150mm x 4.6mm
Particle size: 5µm
Pore size: 140Å

**Conditions:**
Mobile phase: A: 0.1% formic acid in water
B: 0.1% formic acid in acetonitrile

| Time(min.) | %B |
|---|---|
| 0.0 | 20 |
| 1.0 | 20 |
| 6.0 | 80 |
| 8.0 | 80 |

Flow: 1.0mL/min.
Temp.: 30°C
Det.: UV @ 254nm
Instrument: Jasco X-LC

# More Small Particles

For more information on the theory behind small particles, please refer to the complete article, "Explaining the Small Particle Advantage," at **www.restek.com/pharmaceutical**

# Pinnacle™ DB 1.9µm HPLC Columns



## did you **know**?

All phases also available in 3 & 5µm particle sizes using Restek manufactured Pinnacle™ DB Silica!

**Physical Characteristics:**

particle size: 1.9µm
pore size: 140Å
endcap: yes

pH range: 2.5 to 7.5
temperature limit: 80°C

| Length | 2.1mm ID cat.# |
|---|---|
| **Pinnacle™ DB C18 1.9µm Columns** | |
| 30mm | 9414232 |
| 50mm | 9414252 |
| 100mm | 9414212 |
| **Pinnacle™ DB Silica 1.9µm Columns** | |
| 30mm | 9410232 |
| 50mm | 9410252 |
| 100mm | 9410212 |
| **Pinnacle™ DB PFP Propyl 1.9µm Columns** | |
| 30mm | 9419232 |
| 50mm | 9419252 |
| 100mm | 9419212 |
| **Pinnacle™ DB Biphenyl 1.9µm Columns** | |
| 30mm | 9409232 |
| 50mm | 9409252 |
| 100mm | 9409212 |
| **Pinnacle™ DB Aqueous C18 1.9µm Columns** | |
| 30mm | 9418232 |
| 50mm | 9418252 |
| 100mm | 9418212 |
| **Pinnacle™ DB IBD 1.9µm Columns** | |
| 30mm | 9425232 |
| 50mm | 9425252 |
| 100mm | 9425212 |
| **Pinnacle™ DB Cyano 1.9µm Columns** | |
| 30mm | 9416232 |
| 50mm | 9416252 |
| 100mm | 9416212 |
| **Pinnacle™ DB C8 1.9µm Columns** | |
| 30mm | 9413232 |
| 50mm | 9413252 |
| 100mm | 9413212 |

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ISO 9001:2000 cert.# FM80397