

Optimize Selectivity & Efficiency in UHPLC Separations

With More Stationary Phase Choices on 1.9 μ m Pinnacle™ DB HPLC Columns

By Rick Lake, Pharmaceutical Innovations Chemist

- Largest variety of stationary phases for UHPLC.
- Faster analyses, uncompromised chromatography.
- 100% Restek manufactured—from base silica to final packed column.

Since the late 1960s continual advancements have been made in HPLC column technology, and over time the trend has been toward smaller particle sizes. This trend has led us to where we are today—Ultra-High Performance Liquid Chromatography (UHPLC). UHPLC is a milestone in the evolution of LC in that columns packed with <2 μ m particles, used with instrumentation capable of handling the resulting high back pressures, make possible extremely fast and efficient separations. UHPLC is a very powerful tool for today's practicing chromatographer, as it can significantly increase the efficiency of a chromatographic separation. In addition, the wider range of usable flow rates makes high speed separations possible. However, in light of this new technology, it is important that we do not forget the importance of selectivity. In this article, we will review the significance of selectivity in obtaining acceptable resolution and demonstrate how having choices in stationary phase allows you to maximize the benefits of UHPLC.

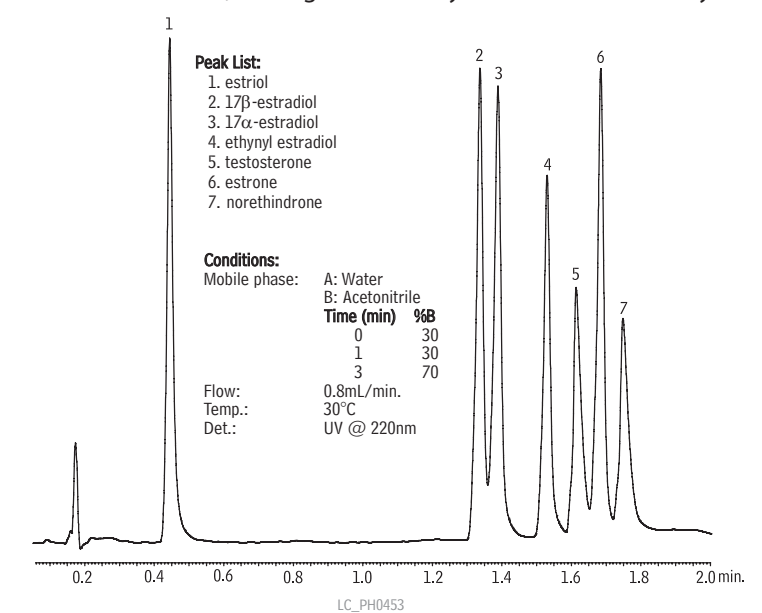
In past articles we have discussed the physical advantages that are driving interest in small particles, mainly the influence of particle size on usable flow rates and peak efficiency. Although small particles have made faster separations possible, selectivity has the greatest effect on resolution. Selectivity, in turn, is governed predominantly by analyte interactions with both the stationary and mobile phases. UHPLC, through the use of small particle columns, does maximize efficiency (e.g. theoretical plates), but the stationary phase is still the most important consideration when attempting to resolve mixtures of compounds. Ideally, a stationary phase that produces optimum selectivity or allows for resolution of compounds in a timely manner should be selected.

Previously, some advantages of selectivity in specific separations have been noted. For example, the use of a unique Biphenyl stationary phase has shown excellent selectivity for aromatic or fused ring compounds. When using the Biphenyl stationary phase and combining it with the heightened efficiencies of the 1.9 μ m Pinnacle™ DB column, we can produce highly selective and fast separations of steroids (Figure 1). A Pinnacle™ DB 1.9 μ m Biphenyl column can separate a test mix of seven hormones in under 2 minutes, a feat not possible through C18 selectivity.

Another example of unique selectivity available on a 1.9 μ m particle size column is the PFP Propyl (pentafluorophenyl propyl) stationary phase for halogenated drug compounds. This phase is very selective and retentive for organohalogens or other compounds containing basic or electronegative functionalities. To demonstrate heightened selectivity for halogenated drug compounds, we assayed a test mix of eight benzodiazepines and two metabolites, a mix commonly assayed on a C18 column, in just over 4 minutes with complete resolution (Figure 2). To get the same level of selectivity from a C18 column, a shallower gradient would be needed, prolonging the analysis time. Since the selectivity of the Pinnacle™ DB 1.9 μ m PFP Propyl column elutes the benzodiazepines in quick succession, a simple gradient still allows for the earlier elution of the more polar metabolites, while maintaining a fast overall run time.

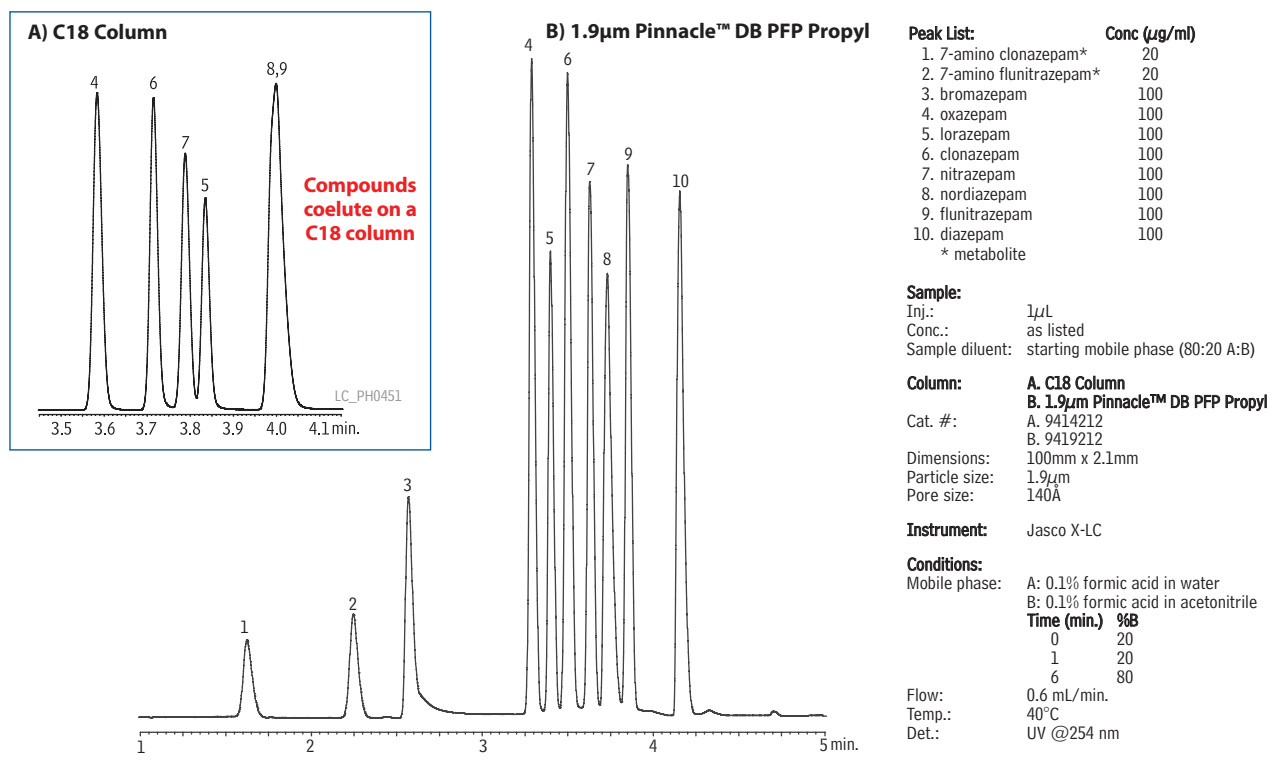
Restek is committed to giving the practicing chromatographer choices, and has therefore sought to deliver the widest selection of stationary phases available with <2 μ m particle sizes. The goal of chromatography is always to resolve compounds of interest in the fastest time possible. By combining the benefits of UHPLC with Restek's complement of unique stationary phase choices, faster separations become a reality.

Figure 1 Restek's 1.9 μ m Pinnacle™ DB Biphenyl columns are highly selective for steroids, making an extremely fast and selective analysis.



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

Figure 2 Fast, selective analysis of benzodiazepines is made possible by combining the speed of UHPLC with the enhanced selectivity of the 1.9 μ m Pinnacle™ DB PFP Propyl column.



1.9 μ m Pinnacle™ DB HPLC Columns

Physical Characteristics:

particle size: 1.9 μ m
 pore size: 140Å
 endcap: yes

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

1.9μm Pinnacle™ DB C18 column, 2.1mm	cat. #
30mm	9414232
50mm	9414252
100mm	9414212
1.9μm Pinnacle™ DB Silica column, 2.1mm	cat. #
30mm	9410232
50mm	9410252
100mm	9410212
1.9μm Pinnacle™ DB PFP Propyl column, 2.1mm	cat. #
30mm	9419232
50mm	9419252
100mm	9419212
1.9μm Pinnacle™ DB Biphenyl column, 2.1mm	cat. #
30mm	9409232
50mm	9409252
100mm	9409212
1.9μm Pinnacle™ Aqueous C18 column, 2.1mm	cat. #
30mm	9418232
50mm	9418252
100mm	9418212

More phases coming soon!

More Small Particles

For more information on the theory behind small particles, please refer to the article, "Explaining the Small Particle Advantage," at

www.restek.com/pharmaceutical



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