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Technical Report

Frit Porosity: An Important Factor to Consider Before Using Sub-2 µm UHPLC Columns

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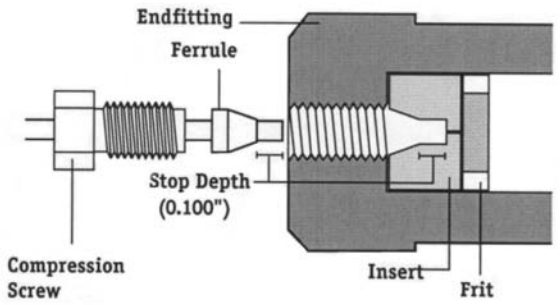
Construction of an HPLC Column

Virtually all columns having an internal diameter greater than 1 mm employ high-quality stainless steel components that consist of a tube with end-fittings and a frit or screen to contain the packing material as shown in Figure 1.

Figure 1. Cross-Section of an HPLC Column Fitting Showing Frit to Contain Particles



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Packing materials always exist as a distribution around a mean value that is reported on the column label. An example of a typical distribution for a porous particle is shown in Figure 2 for the size 3-5 µm packing material. The example selected has a standard deviation of 19% and represents about the smallest porous particle that can be safely contained with a 2 µm frit or screen. This specific example would likely be labeled as 3.5 or 4 µm. Particles in the 3 µm or smaller range generally require frit porosity of 0.5-1 µm, and particles in the sub-2 µm range require extremely fine frits with porosity of about 0.2 µm.

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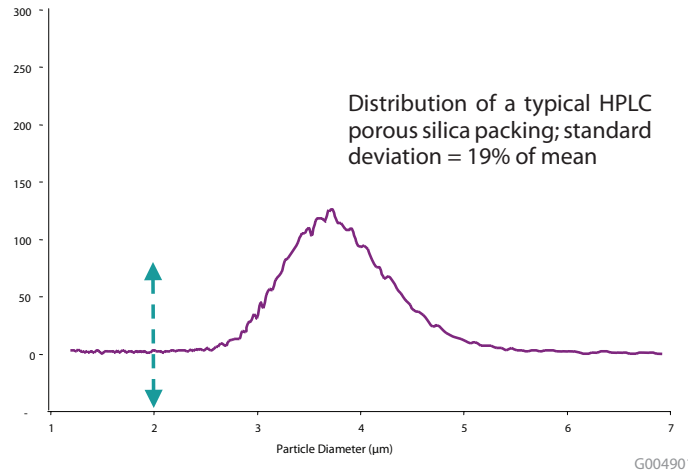
Figure 2. Normal Distribution of Particles for a Porous HPLC Packing in the 3-5 µm Range



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Why Frit Porosity is Important to Column Ruggedness

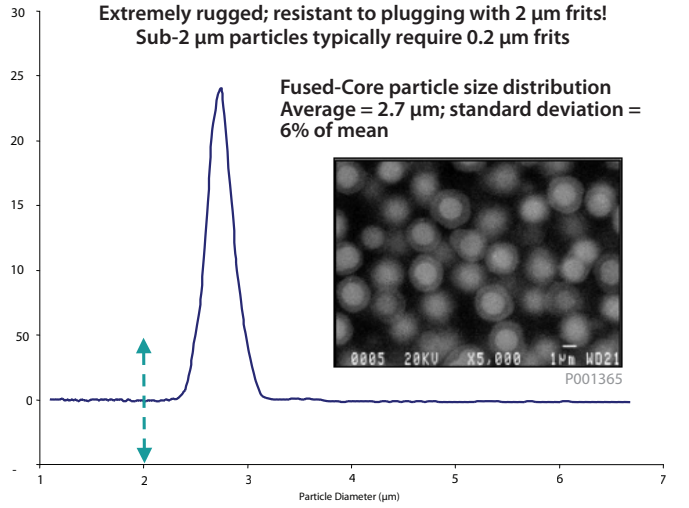
As a critical component of the HPLC system design, the frit should be just small enough to fully contain the finest packing particles but not so small that it plugs prematurely from accumulation of particles from exterior sources. Majors (1) has reported that the most common HPLC column complaint is development of high backpressure due to particle contamination at the inlet frit. A main source of particles larger than the frit openings is the sample; however, particles can also be introduced by the mobile phase and instrument components. Column pre-filters and guard columns can provide some column protection, but early development of backpressure is a major inconvenience when it occurs. The use of UHPLC columns with sub-2 µm particles requires extremely careful sample preparation and pre-column filters to protect columns from development of high backpressure.

High Porosity of Ascentis® Express UHPLC Columns

Ascentis Express columns employ 2 µm frits and are very resistant to plugging. The reason for this is two-fold. First, the Fused-Core™ particles are larger with an average diameter of 2.7 µm even though their unique design can generate efficiencies and performance very comparable to sub-2 µm particle columns. Secondly, the denser particles can be manufactured with a much narrower size distribution as shown in Figure 3. The standard deviation of 6% is much less than that of the

typical porous particle. Column ruggedness is enhanced by the fact that denser particles are easier to pack into a uniform bed that doesn't settle in use. Even a small amount of bed settling will create a void that is large enough to destroy the very high efficiency of any UHPLC column. An additional factor in ruggedness is provided by the Fused-Core bed itself, which has much higher porosity and resists plugging better than a bed of sub-2 µm particles.

Figure 3. Normal Distribution for 2.7 µm Fused-Core™ Particles Used in Express HPLC Columns



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Proven in Use

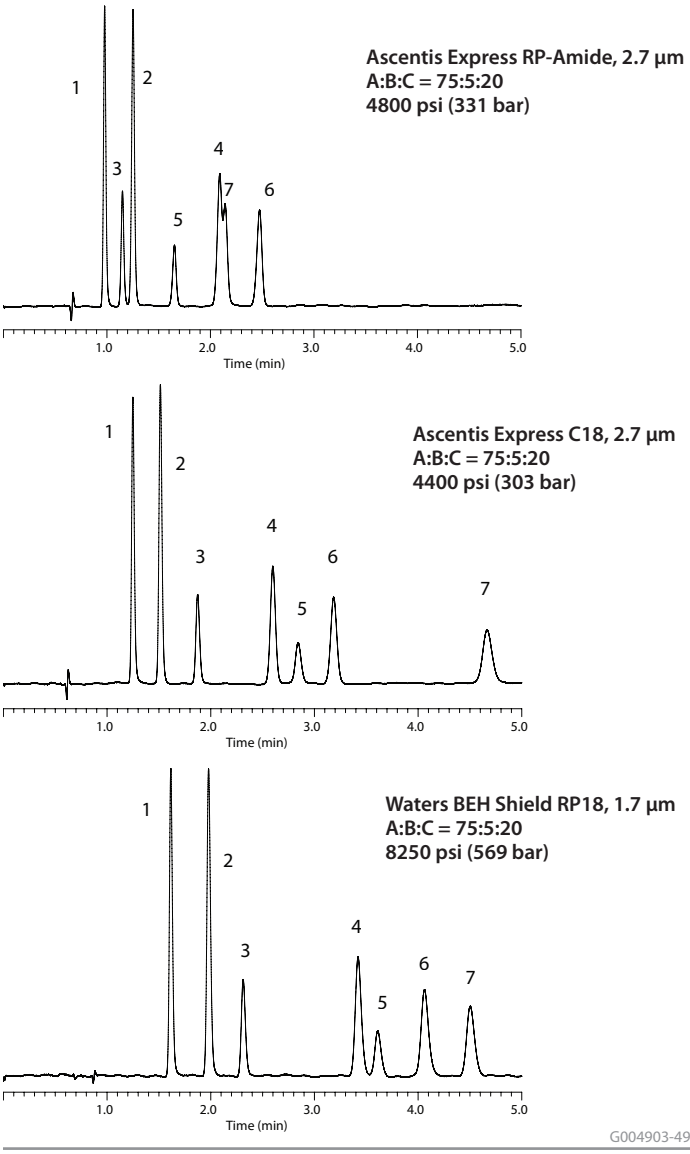
After several years of experience with Express columns, users have reported impressive column ruggedness and reproducibility even under hard use with many thousands of sample injections. A recent publication by Mallett (2) compares Express columns very favorably with various sub-2 µm and other columns, citing important advantages of Fused-Core particles and Express columns in low operating pressure and extreme ruggedness over thousands of protein hypenate precipitated plasma extracts.

The example of Express column performance in Figure 4 highlights the extra selectivity of Express RP-Amide over Express C18 for phenolic natural products. Note that Express RP-Amide operates at about half the pressure of a Waters® BEH Shield (carbamate) while providing similar retention and separation under the same mobile phase conditions.

Figure 4. Extra Selectivity of Ascentis Express RP-Amide over Ascentis Express C18 and Comparison to Sub-2 µm Competitor

column: 10 cm x 2.1 mm I.D.
mobile phase A: 20 mM phosphoric acid, pH 2
mobile phase B: water
mobile phase C: acetonitrile
flow rate: 0.3 mL/min.
temp.: 35 °C
det.: UV @ 270 nm

1. Resorcinol
2. Catechol
3. 2-Methylresorcinol
4. 4-Methylcatechol
5. 2,5-Dimethylresorcinol
6. 3-Methylcatechol
7. 4-Nitrocatechol



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Trademarks

Ascentis, Supelco — Sigma-Aldrich Biotechnology LP
Fused-Core – Advanced Materials Technology, Inc.
Waters BEH Shield — Waters Corporation

References

1. R. Majors, LC/GC, 15, 1008 (1997).
2. D. Mallett, Journal of Pharmaceutical and Biomedical Analysis, 49 (2009) 100-107.

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