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SUPELCO Analytical

Application Note 183

Improving HPLC Performance: Relationship between particle size, column efficiency and column pressure

One of the most important operating parameters in High Performance Liquid Chromatography (HPLC) is pressure drop across the column. Performance is normally assessed by how well and how fast the target components in a sample can be separated as they pass through a packed column and are detected as a chromatogram. Degree of separation is quantitatively measured from the chromatogram by the Resolution Equation (Figure 1), where N is efficiency or plate number, k is retention factor calculated relative to the column void volume, and α is selectivity factor calculated from the ratio of retention factors. A resolution of 1.5 is considered to be baseline, and many labs prefer to achieve a higher number to allow for changes caused by random variations in sample and method conditions.

Figure 1. Resolution Equation

$$R = \frac{\sqrt{N}}{4} \times \frac{k}{k+1} \times \frac{\alpha - 1}{\alpha}$$

HPLC columns are packed with a distribution of particles. A distribution average is normally stated in the column specification (i. e. 2.7 µm, 3 µm, 5 µm, etc.). Column operating pressure is at the heart of the current debate about whether traditional HPLC instruments, which are limited to about 400 bar (6,000 psi) should be replaced with systems having higher pressure ratings, which are often referred to as Ultra HPLC (UHPLC) designs. The higher pressure requirement comes from the trend toward use of smaller particles to achieve higher column efficiency. For porous particles, efficiency has the following relationship to particle diameter if the column has been prepared with a uniform bed.

$$N \approx L/2d_P$$

where N is efficiency, L is column length and d_P is the particle diameter.

For any particles, pressure drop can be estimated by the following relationship,

$$P = \eta v L/d_P^2$$

where **η** refers to mobile phase viscosity (which changes with column temperature), **v** is mobile phase velocity (which is controlled by flow rate) and L is column length. Note also that pressure across the column is inversely related to the column diameter squared. Column pressure increases much faster than efficiency as particle size is reduced so it is highly desirable to get as much performance from a given diameter particle as possible.

An important relationship called a van Deemter plot (Figure 2) describes column efficiency in terms of plate height (H) as a function of flow velocity of the mobile phase solvent through the column. H is the column length that is equivalent to one plate and allows efficiency to be compared for different column lengths. Velocity is normally calculated by dividing column length, L, by the time it takes for an unretained peak to appear the solvent front.

$$v = L/t_M$$

The advantages of smaller particles are clearly shown in Figure 2 - they have smaller H at the optimum flow (where the minimum occurs in the plot), which results in higher N for a given column length. Also, smaller particles do not lose performance as rapidly when flow velocity is increased; however, the optimum flow for smaller particles does increase to make the required operating pressure even higher. The increase from 5 µm to 3 µm is about 20-25%. The practical consequence is that UHPLC columns should be operated at slightly higher flows to achieve the best efficiency and overall performance. When need for higher flow velocity is combined with the already high-pressure drop of sub-2 µm particle columns, the use of traditional HPLC instruments with these columns becomes difficult or impossible.

Figure 2. Graph illustrating reduced plate height as a function of mobile phase velocity for several particle sizes

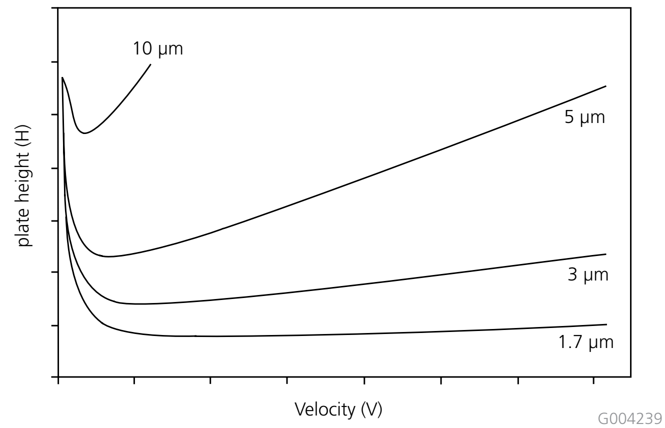


Table 1. Compares typical operating parameters for some modern columns in a popular, standard column length of 10 cm x 4.6 mm at constant linear velocity

Particle Size	psi	N
1.8	6000	27,000
2.5	3090	20,000
3	2120	16,500
5	770	10,000
10	190	5,000

The practical consequence of Table 1 is that traditional HPLC instruments with operating limits of 400 bar cannot operate reliably with sub-2 µm particle columns. With so many HPLC systems currently installed, is there a solution to provide high speed and high resolution HPLC chromatography without the high backpressure of sub-2 µm particle columns?

Introducing Ascentis® Express and Fused-Core™ Technology

Designed for high speed and high resolution, Ascentis Express columns provide a breakthrough in HPLC performance. Based on unique Fused-Core particle technology, Ascentis Express provides the high-efficiency based benefits of sub-2 µm particles, but at much lower backpressures. Due to the high efficiencies at low back pressures, Ascentis Express can provide HPLC performance that was previously unattainable on traditional LC systems.

The Fused-Core particle consists of a 1.7 µm solid core and a 0.5 µm porous shell. Major benefits of the Fused-Core particle are uniform bed and small diffusion path (0.5 µm) compared to conventional fully porous particles. The shorter diffusion path reduces dispersion of solutes and minimizes peak broadening, especially for larger molecules. In fact, Ascentis Express columns are able to achieve efficiencies similar to that obtained with sub-2 µm particle columns, even though the backpressures are only 50% of that observed under similar conditions with sub-2 µm particles. This means that Ascentis Express can turn almost any LC system into a Fast HPLC workhorse for your lab.

Traditional HPLC systems can use 2.7 µm Ascentis Express columns to improve separation performance in much the same fashion that 3 µm columns are now employed. Compared to 3 µm, Ascentis Express columns with half of the length will provide the same resolution in about half the time, or columns with the same length will provide more resolution in the same time. Columns as long as 10 or even 15 cm can be installed without exceeding component pressure limits. By contrast, 1.7 or 1.8 µm columns longer than 5 cm will usually exceed 400 bar operating limits and require special instrumentation. It should be relatively simple for users to achieve significant performance enhancement with Express columns and traditional instruments as long as 4.6 mm I.D. columns are employed. The 3 mm and 2.1 mm I.D. Ascentis Express columns will require more optimization for best results because peak volumes become extremely small and too similar to the internal volume of the instrument. Pressure requirement will be the same for a given column length because flow rate with column I.D. is reduced to maintain constant flow velocity (flow velocity is independent of column I.D.).

Figure 3 compares the resolution of a five-component sample on 25 cm, 5 µm C18 and 10 cm Ascentis Express C18 columns. Each column has approximately the same number of theoretical plates and hence the same resolving power. However the shorter Ascentis Express column delivers this separation in a much shorter time, in this case less than one-fourth the time as the 25 cm column.

As a final word of caution about high-pressure operation, keep in mind that typical, popular PEEK fittings may not hold at pressure above about 200 bar unless they are of a special, new design. Figure 4 shows an example of how performance first degrades before a typical PEEK fitting fails at about 200 bar. Degradation may be caused by slipping and possible stretching of the polymer tubing. Special high pressure fittings are available.

Figure 3. Increase Sample Throughput by Using Ascentis Express

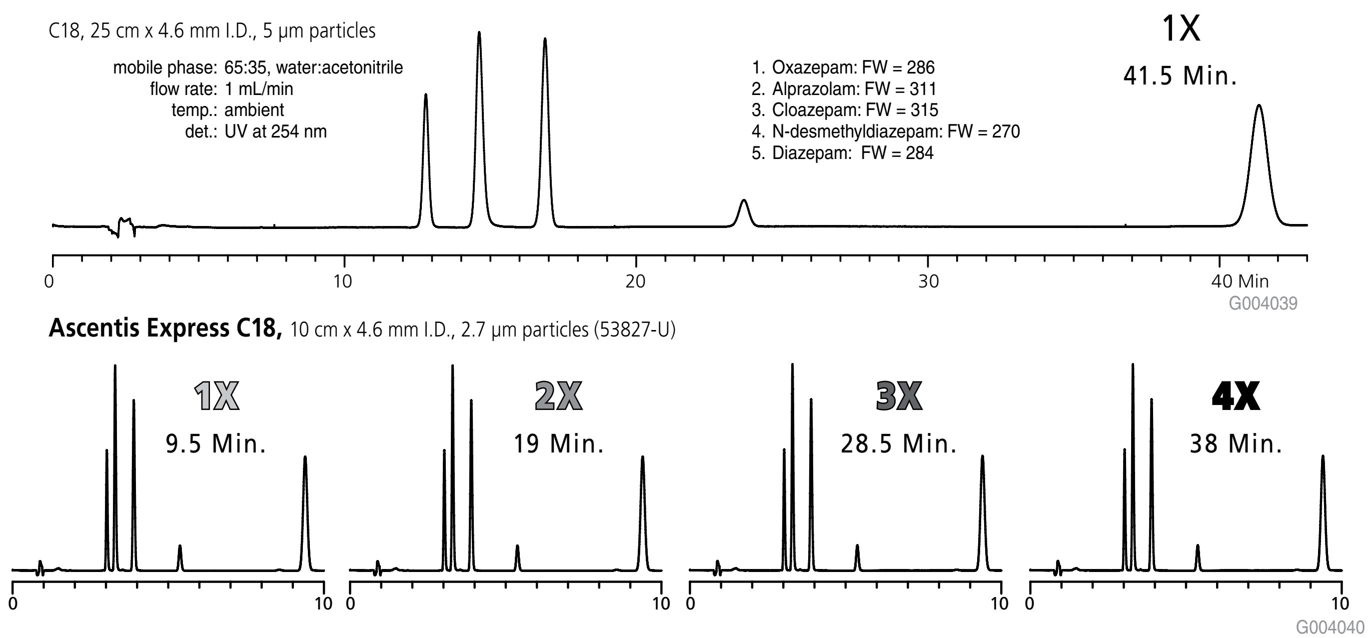
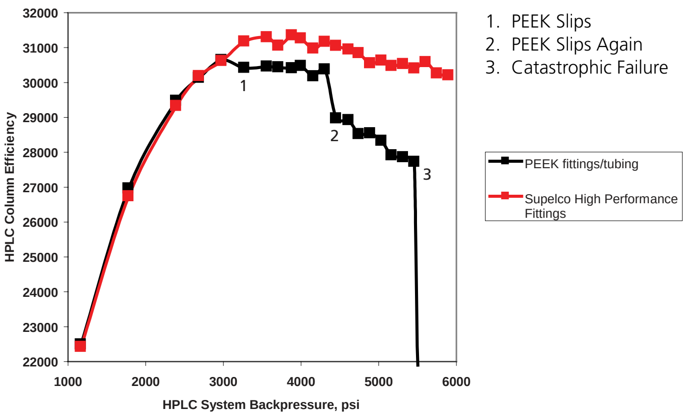


Figure 4. Supelco High Performance Fittings versus Standard PEEK Fittings



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Ordering Information

Ascentis Express Columns

Column Dimensions	C18	C8	HILIC
3 cm x 2.1 mm I.D.	**53802-U**	**53839-U**	**53933-U**
5 cm x 2.1 mm I.D.	**53822-U**	**53831-U**	**53934-U**
7.5 cm x 2.1 mm I.D.	**53804-U**	**53843-U**	**53938-U**
10 cm x 2.1 mm I.D.	**53823-U**	**53832-U**	**53939-U**
15 cm x 2.1 mm I.D.	**53825-U**	**53834-U**	**53946-U**
3 cm x 3.0 mm I.D.	**53805-U**	**53844-U**	**53964-U**
5 cm x 3.0 mm I.D.	**53811-U**	**53848-U**	**53967-U**
7.5 cm x 3.0 mm I.D.	**53812-U**	**53849-U**	**53969-U**
10 cm x 3.0 mm I.D.	**53814-U**	**53852-U**	**53970-U**
15 cm x 3.0 mm I.D.	**53816-U**	**53853-U**	**53972-U**
3 cm x 4.6 mm I.D.	**53818-U**	**53857-U**	**53974-U**
5 cm x 4.6 mm I.D.	**53826-U**	**53836-U**	**53975-U**
7.5 cm x 4.6 mm I.D.	**53819-U**	**53858-U**	**53977-U**
10 cm x 4.6 mm I.D.	**53827-U**	**53837-U**	**53979-U**
15 cm x 4.6 mm I.D.	**53829-U**	**53838-U**	**53981-U**

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