Chromatography Solutions

Knowledge note #0012

Understanding the Relationship between Particle Size, Performance and Pressure

INTRODUCTION

Chromatographers are inundated with vendor information on different particle sizes and silica platforms, which can lead to confusion as to what is the best solution for their application. It is important to understand the benefits of different particle sizes, and how the chromatography obtained is affected. There are several well documented benefits to using sub-2 µm silica particles, and this Knowledge Note will discuss some of these, as well as the relationship between using smaller particles and their effect on performance and column efficiency.

PARTICLE SIZE AND EFFICIENCY

Column efficiency is dependant on the particle size and one of the key benefits of moving towards smaller particles is the increased efficiency provided. It is therefore important to know how to compare particle sizes and what efficiency means in practice.

The efficiency provided by different particle sizes can be compared by examining their respective van Deemter curves, which plot height equivalent to a theoretical plate (HETP) vs linear velocity in mm/sec. The van Deemter plot is composed of three critical terms: eddy diffusion, diffusion coefficient and mass transfer coefficient (for further details, please see AKN0010). Smaller particles have smaller diffusion paths which increase mass transfer, which has the potential to reduce plate height, and therefore increase efficiency.

The data can also be examined by plotting efficiency vs flow rate, as shown in Figure 1. As demonstrated, each particle size has a different optimum flow rate, at which maximum separation efficiency is generated. The smaller particle size has a broader optimum flow rate range, at much higher flows than larger particle sizes.

Some of the key benefits for decreasing particle size include:

- **Increased column efficiency**
  As shown in equation 1, efficiency is inversely proportional to particle size. This therefore means, as

particle size decreases, efficiency increases.

$$N \approx \frac{L}{2d_P} \qquad \text{Equation 1}$$

- **Shorter analysis time**
  The higher efficiency of smaller particles means that shorter columns can be used, without losing resolution. Additionally, higher flow rates can be used to further decrease analysis times.

- **Increased sample throughput**
  With the increased flow/shorter analysis time, the sample throughput will increase, therefore improving laboratory productivity.

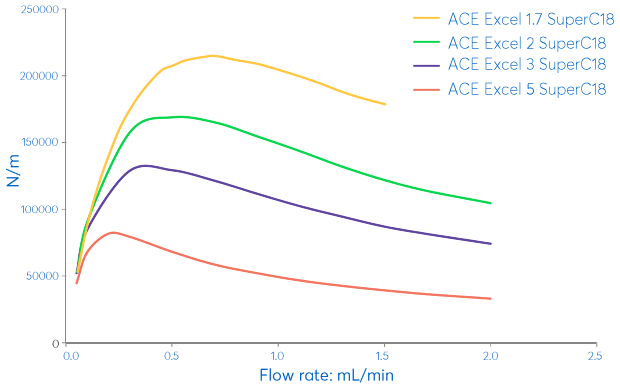

Figure 1: Plot of efficiency, N, against flow rate for the different Avantor® ACE® particle sizes packed in 50 x 2.1 mm columns.

It is important to note that when decreasing particle size there is a trade-off with increased back pressure (Figure 2). It is important to monitor the back pressure to avoid exceeding the pressure rating of the LC system and column. The pressure drop of any particle size can be estimated using equation 2:

$$P = \frac{\eta v L}{d_P^2} \qquad \text{Equation 2}$$

Where η is mobile phase viscosity, υ is mobile phase velocity, L is column length and d_P is particle size.

HOW DOES THIS AFFECT CHROMATOGRAPHY?

The example shown in Figure 3 shows the potential benefits of 1.7 µm particles, for either increased analysis speed, or increased resolution. In this example, a paracetamol (and related substances) separation was originally done using an ACE 5 C18, 150 x 4.6mm column

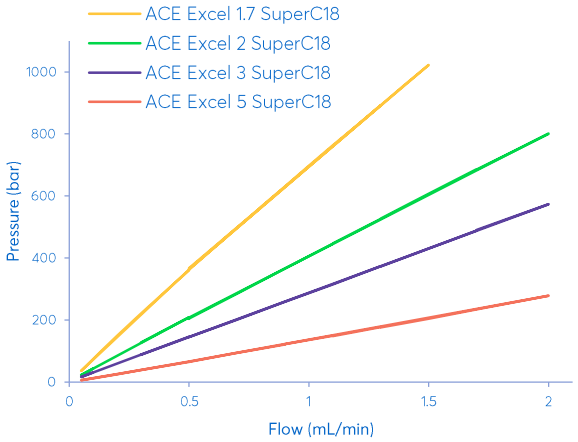

Figure 2: Pressure vs flow rate for different particle sizes

(Figure 3a). The separation was then translated to an ACE Excel 1.7 C18, 50 x 3.0 mm to obtain a faster analysis time (Figure 3b). The run time decreased by 65%, whilst approximately maintaining the resolution (Table 1). The separation was also translated to an ACE Excel 1.7 C18, 100 x 3.0 mm column, to increase both peak capacity (P_C) and resolution (Figure 3c).

The 1.7 µm particle can also be used to achieve ultra-resolution separations, as shown in Figure 4. This approach is useful for complex samples such as the natural product, Ginkgo Biloba. In this example, two columns were coupled in series to increase the effective bed length, thereby increasing peak capacity and resolution. The highlighted region demonstrates how both sensitivity and resolution are increased, revealing more sample complexity.

CONCLUSION

This Knowledge Note has discussed the benefits of using columns packed with smaller particles. The importance of considering the associated increase in back pressure has been highlighted. The use of smaller particle sizes to achieve increased analysis speed, increased resolution and for ultra-resolution of complex samples has been demonstrated.

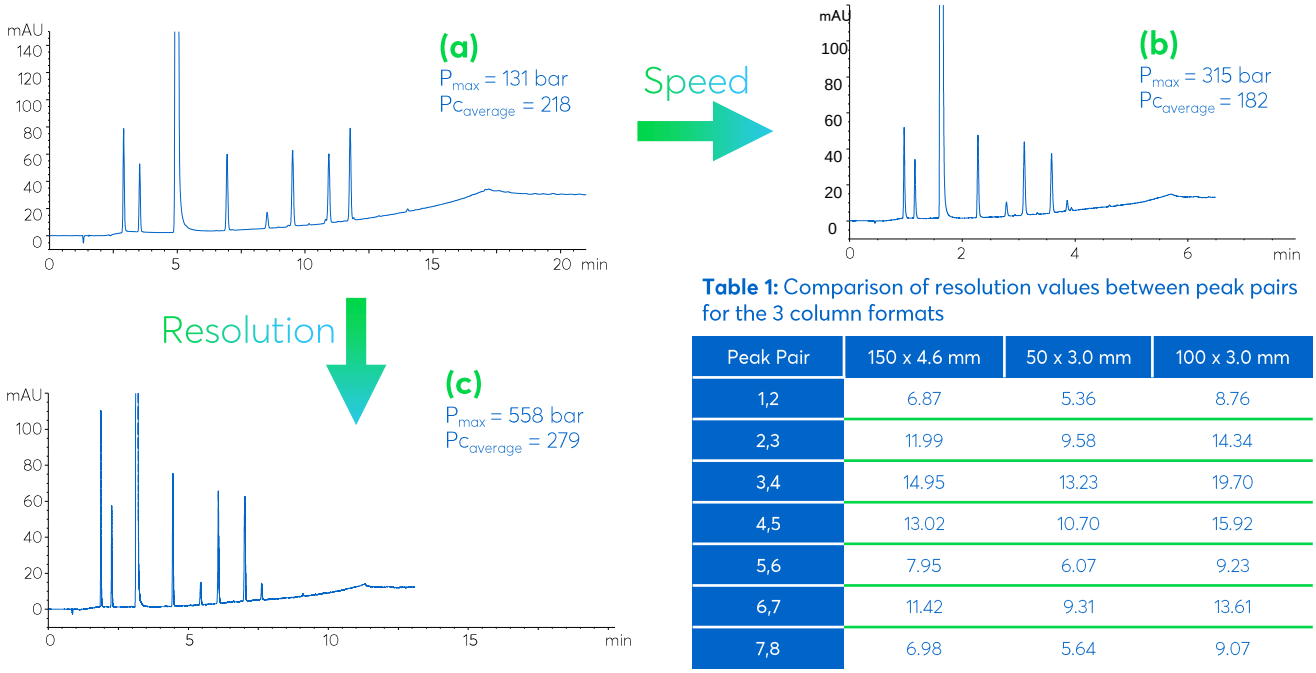

Table 1: Comparison of resolution values between peak pairs for the 3 column formats

Peak Pair	150 x 4.6 mm	50 x 3.0 mm	100 x 3.0 mm
1,2	6.87	5.36	8.76
2,3	11.99	9.58	14.34
3,4	14.95	13.23	19.70
4,5	13.02	10.70	15.92
5,6	7.95	6.07	9.23
6,7	11.42	9.31	13.61
7,8	6.98	5.64	9.07

Figure 3: Paracetamol and related substances, gradient separation: (a) ACE 5 C18, 150 x 4.6 mm, 1 mL/min (b) ACE Excel 1.7 C18, 50 x 3.0 mm, 0.51 mL/min, (c) ACE Excel 1.7 C18 100 x 3.0 mm, 0.51 mL/min.

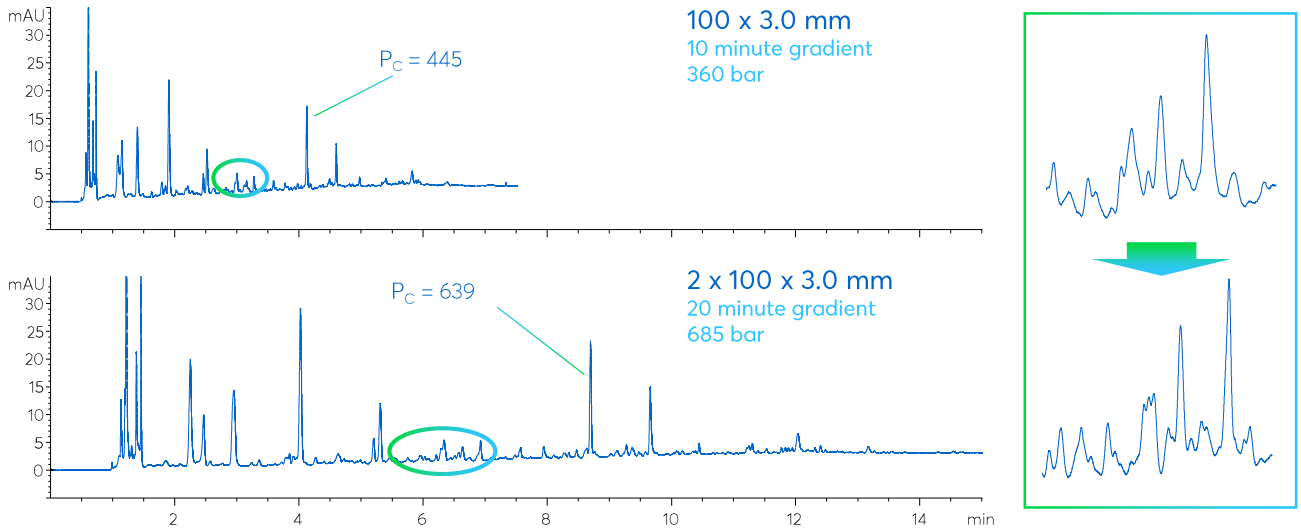

Figure 4: Ginkgo Biloba ultra resolution example on ACE Excel 1.7 C18-PFP 100 x 3.0 and 200 x 3.0 mm column formats.