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Technical Report

Keys to High Speed HPLC with Isocratic Mobile Phases

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Introduction

Speed refers to the time required to run a complete assay and introduce a new sample (for gradient assays, speed must also include the time required for system re-equilibration). Important goals in HPLC are increasing speed and maintaining resolution. Resolution is controlled by three column performance parameters as shown in Equation 1.

$$R_S = \frac{\sqrt{N}}{4} \cdot \frac{k}{1+k} \cdot \frac{\alpha-1}{\alpha} \qquad (1)$$

Resolution refers to the amount of space between peaks. Best analytical performance is achieved when peaks are completely separated ($R_S = 1.5$ is defined as baseline resolution). Retention factor (k) and selectivity factor (α) do not change with column length; however, efficiency (N) decreases with length unless the particle size is also decreased. Installing a shorter column containing smaller particles can increase speed without degrading resolution because the same number of plates can be maintained. Table 1 shows the history of the development of HPLC particles. Table 2 shows some popular column lengths for several particle types with typical numbers of plates for each column if it is in new condition. Achieving the same number of plates using shorter columns with smaller particles is one key to higher speed under isocratic conditions.

Table 1. Evolution of HPLC Particles

| 1970s	1980s	1990s	Modern
10 µm (Porous)	5 µm (Porous)	3 µm (Porous)	Fused-Core™ and sub-2 µm

Table 2. Comparison of Three Column Particles

Particle Size (µm)	Column Length (mm)	Bed Efficiency (N/mm)	Total Number of Plates (N)
5	150	100	15,000
5	100	100	10,000
5	50	100	5,000
3	100	150	15,000
3	50	150	7,500
3	30	150	4,500
2.7 Fused-Core	100	250	25,000
2.7 Fused-Core	50	250	12,500
2.7 Fused-Core	30	250	7,500
2.7 Fused-Core	20	250	5,000

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HPLC speed has been further improved by operating shorter columns and smaller particles at higher flow rates; however, considerable resolution was sacrificed due to loss of efficiency even with popular 3 µm particles. The reason for this can be seen in Figure 1 where van Deemter plots for different particle sizes are compared.

Figure 1. van Deemter Plots (Efficiency versus Flow Velocity) and Pressure Plots for Different Particle Types
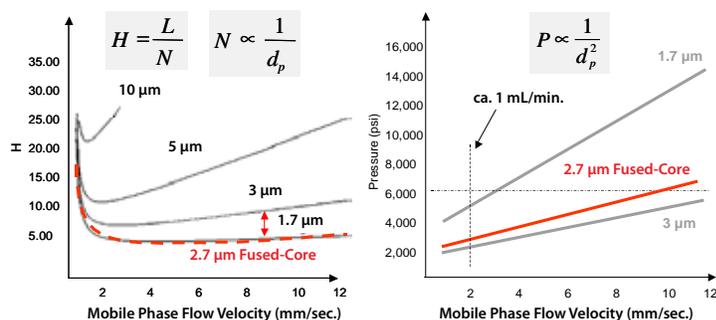
Since the slope of the van Deemter plot decreases dramatically with reduction in particle size or change to a Fused-Core design, both H and N remain constant even at higher flows. This flat van Deemter plot allows an increase in flow to be combined with a reduction in column length to generate much higher separation speed without significant sacrifice in resolution.

Decreasing Column Length for Speed

Figure 2 shows an example of how a separation achieved with column geometry of 150 x 3 mm, 5 µm particles and 0.4 mL/min. (about 2 mm/sec. flow velocity) can be transferred to much shorter column geometry of 50 x 3 mm when higher performance Fused-Core particles are employed with a similar mobile phase. In this case, the Fused-Core particles have about three times the efficiency per unit length of 5 µm particles, so the same separation can be achieved with almost one third the column length. Note that only one third as much solvent is needed because solvent use is directly proportional to column length. This shorter length creates an immediate speed improvement by a factor of three when operated at the same flow velocity. The Fused-Core column was operated at a slightly higher flow rate of 0.6 mL/min. (flow velocity of 3 mm/sec.) so the total increase in speed was four-fold.

Increasing Flow Rate for Speed

Figure 2 also shows the achievement of even more speed and throughput by increasing flow rate for the 50 x 3 mm column to 1.2 mL/min. (flow velocity of 6 mm/sec.). The relationship that defines speed for an isocratic assay is shown in Equation 2,

$$Speed = L/u \, (1 + k) \qquad (2)$$

where L is column length and u is flow velocity (usually measured from the retention time for an unretained peak). In this example, a 50 mm column operated at a flow velocity of 6 mm/sec. yields a speed of 8.3 sec. for k = 0 (solvent front or void volume) so a separation with a k value of 10 or less can be completed in about 90 seconds (see Equation 2). In the 50 x 3 mm example, all peaks elute between k = 0 and k = 2 so the separation is complete in about 25 seconds. Note that very little resolution is sacrificed, which allows peak integration accuracy to be maintained even at high flow velocity.

Conclusions

Modern HPLC columns, such as those which use Fused-Core particles, have several advantages that allow dramatic improvements in analysis speed:
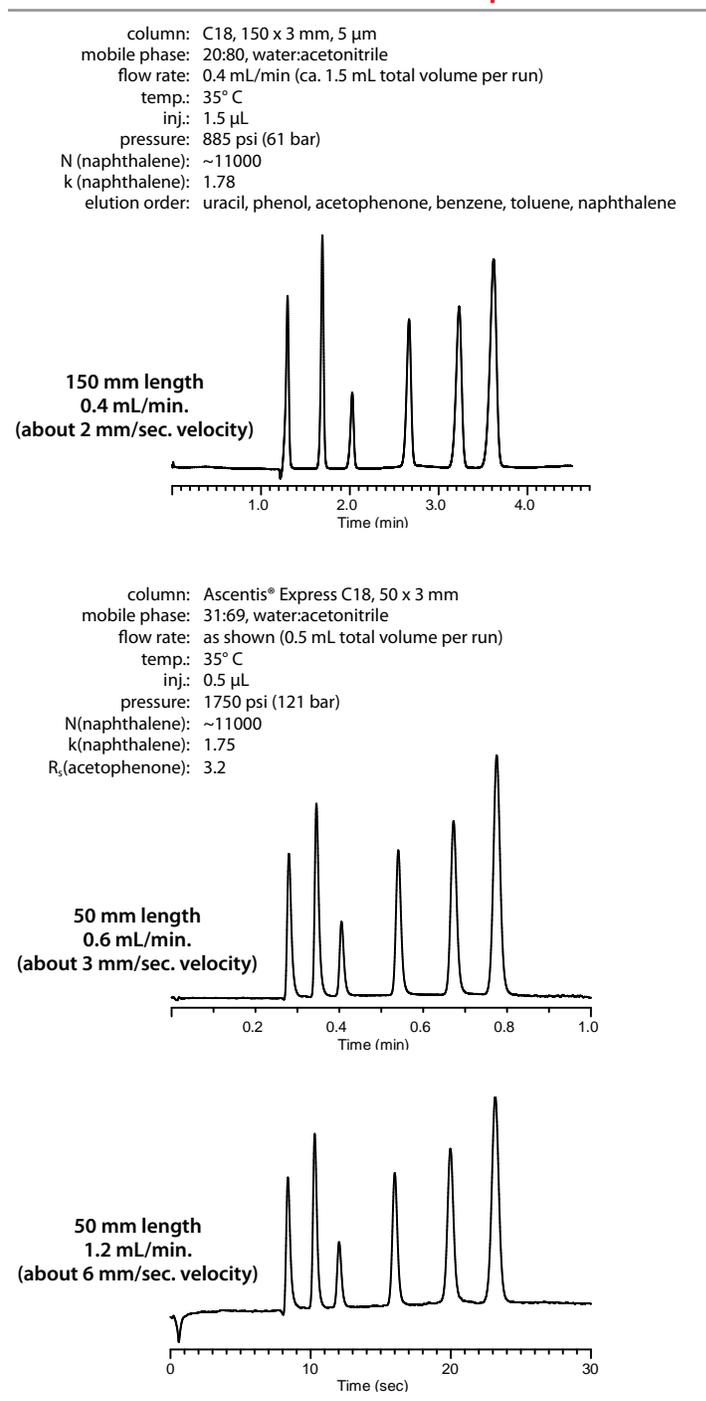
1. Fused-Core and sub-2 µm porous particles have higher efficiency per unit length so that shorter columns can accomplish the same separation as longer columns with 5 µm or 3 µm porous particles.

2. Fused-Core 2.7 µm particles achieve the same performance as sub-2 µm particles at pressures which are only slightly higher than 3 µm particles.

3. Both Fused-Core and sub-2 µm particles can be operated at higher flows without losing efficiency and resolution; however, the lower pressure drop of Fused-Core allows higher flow velocities at lower operating pressures.

When isocratic mobile phase conditions are replaced by solvent strength gradients, significant increase in separation speed can only be achieved by increasing the gradient slope or rate of change in %B (decreasing the gradient time). If column length is decreased, gradient time should also be decreased by the same proportion; if flow rate is increased, gradient time should also be decreased by the same proportion.

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Fused-Core — Advanced Materials Technology, Inc.

Figure 2. Example of using Shorter Columns and Higher Flows with Smaller Particles to Increase Speed

column:	C18, 150 x 3 mm, 5 µm
mobile phase:	20:80, water:acetonitrile
flow rate:	0.4 mL/min (ca. 1.5 mL total volume per run)
temp.:	35° C
inj.:	1.5 µL
pressure:	885 psi (61 bar)
N (naphthalene):	~11000
k (naphthalene):	1.78
elution order:	uracil, phenol, acetophenone, benzene, toluene, naphthalene

150 mm length
0.4 mL/min.
(about 2 mm/sec. velocity)

column:	Ascentis® Express C18, 50 x 3 mm
mobile phase:	31:69, water:acetonitrile
flow rate:	as shown (0.5 mL total volume per run)
temp.:	35° C
inj.:	0.5 µL
pressure:	1750 psi (121 bar)
N(naphthalene):	~11000
k(naphthalene):	1.75
R_s(acetophenone):	3.2

50 mm length
0.6 mL/min.
(about 3 mm/sec. velocity)

50 mm length
1.2 mL/min.
(about 6 mm/sec. velocity)

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