Introduction

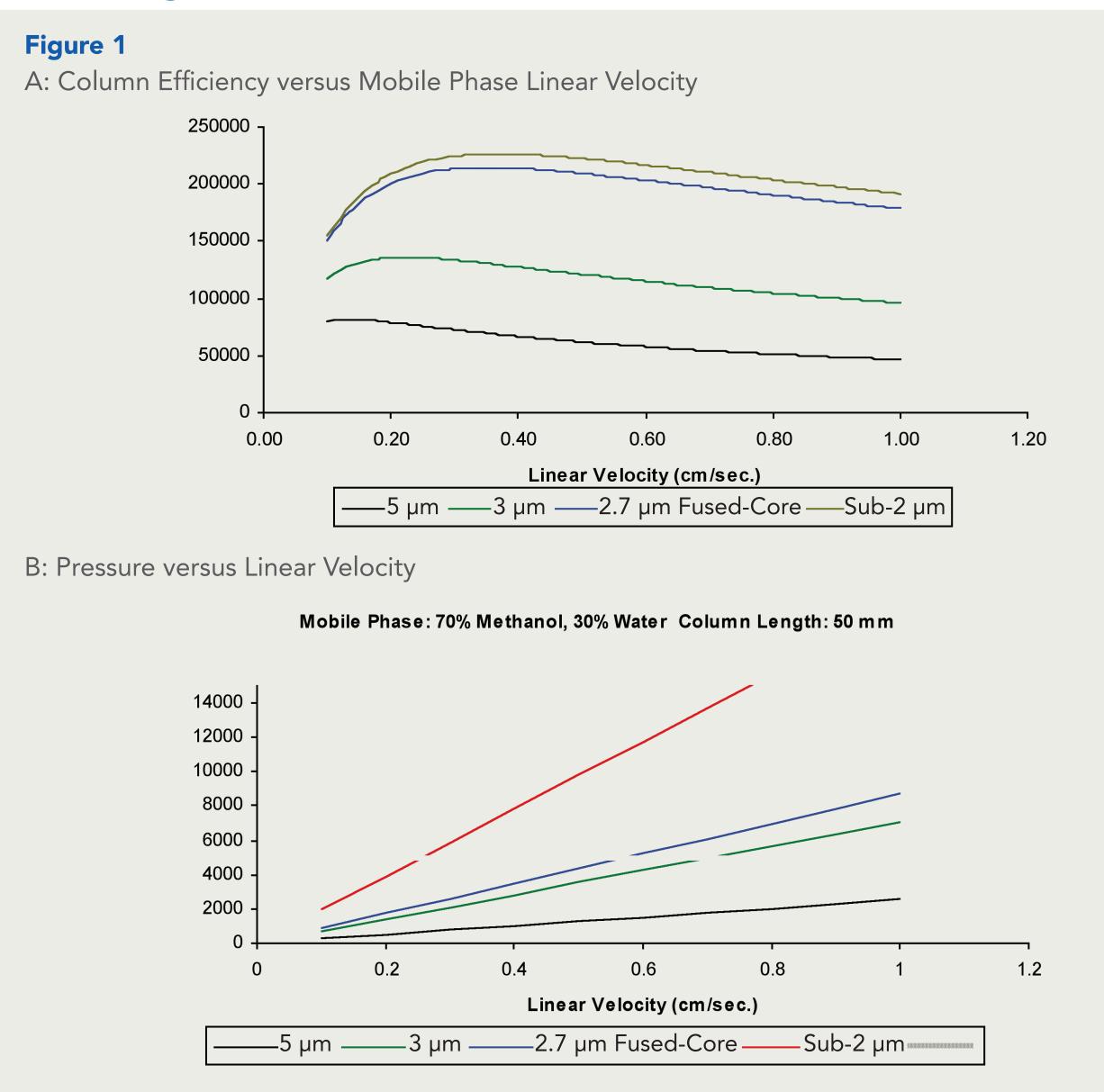
UHPLC is becoming increasingly popular because of its ability to achieve very fast separations or resolution of very difficult-to-separate components. Until recently, it has not been possible to obtain UHPLC performance with conventional HPLC instruments because of the excessive extra column volume in conventional equipment or because of pressure limitations that prevent the full utilization of UHPLC columns.

The introduction of a new type of UHPLC column based on Fused-Core particle technology now offers the opportunity to achieve UHPLC-like performance with conventional equipment. UHPLC columns packed with Fused-Core particles operate effectively within the pressure limits of most conventional HPLCs. However, in order to deliver UHPLC performance conventional HPLC equipment must be modified to reduce extra column volume, and detector response time and data acquisition rate must also be set at values appropriate for the particular Fused-Core column dimensions being used.

This paper will explore the instrument parameters that limit the performance of UHPLC columns when used with conventional HPLC equipment, and will offer recommendations for modifying conventional HPLC equipment and method parameters to achieve UHPLC-like performance.

Three barriers to achieving UHPLC performance with conventional HPLC equipment

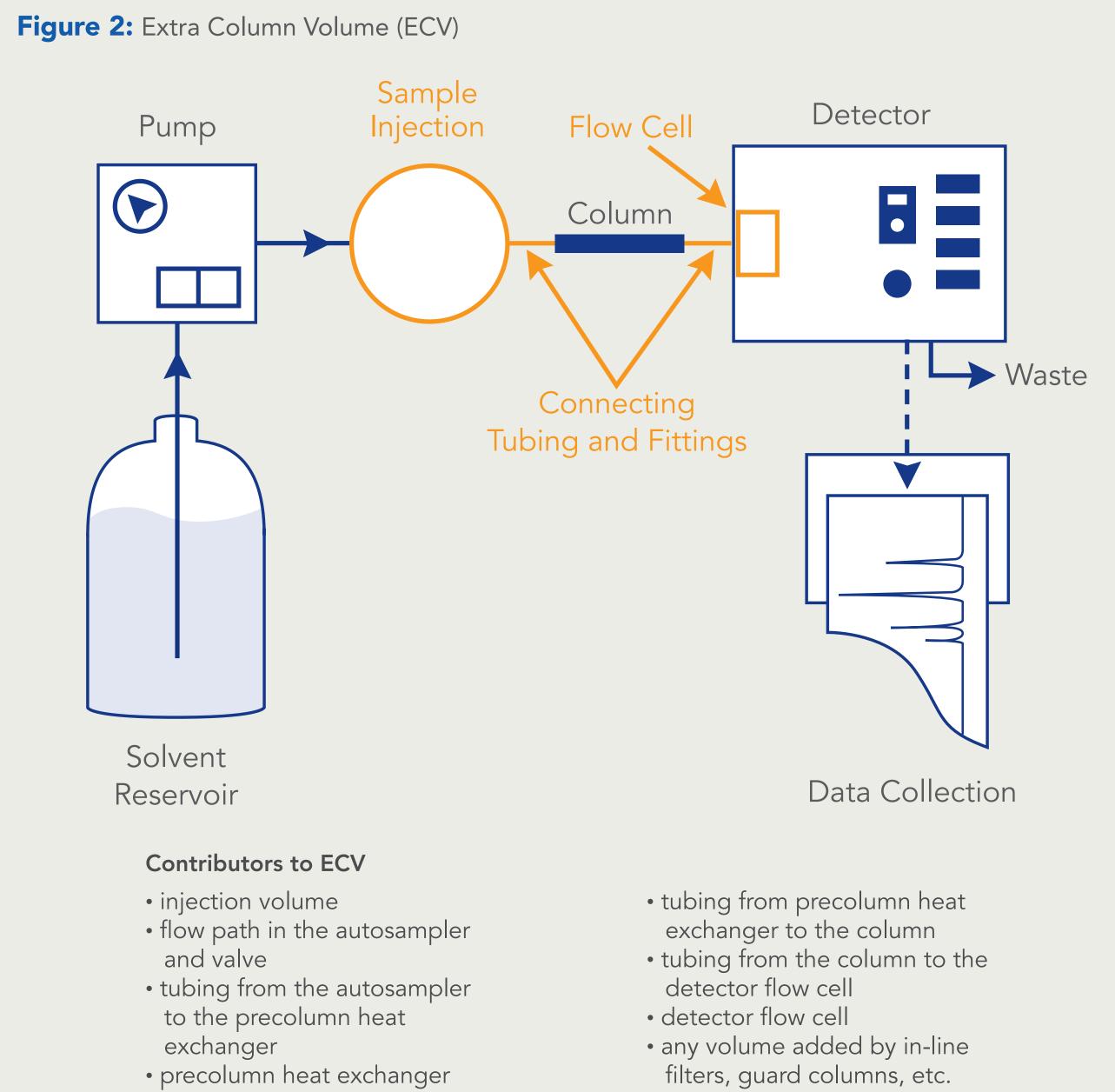
• Pressure • Extra column volume • Detector time constant and data collection rate

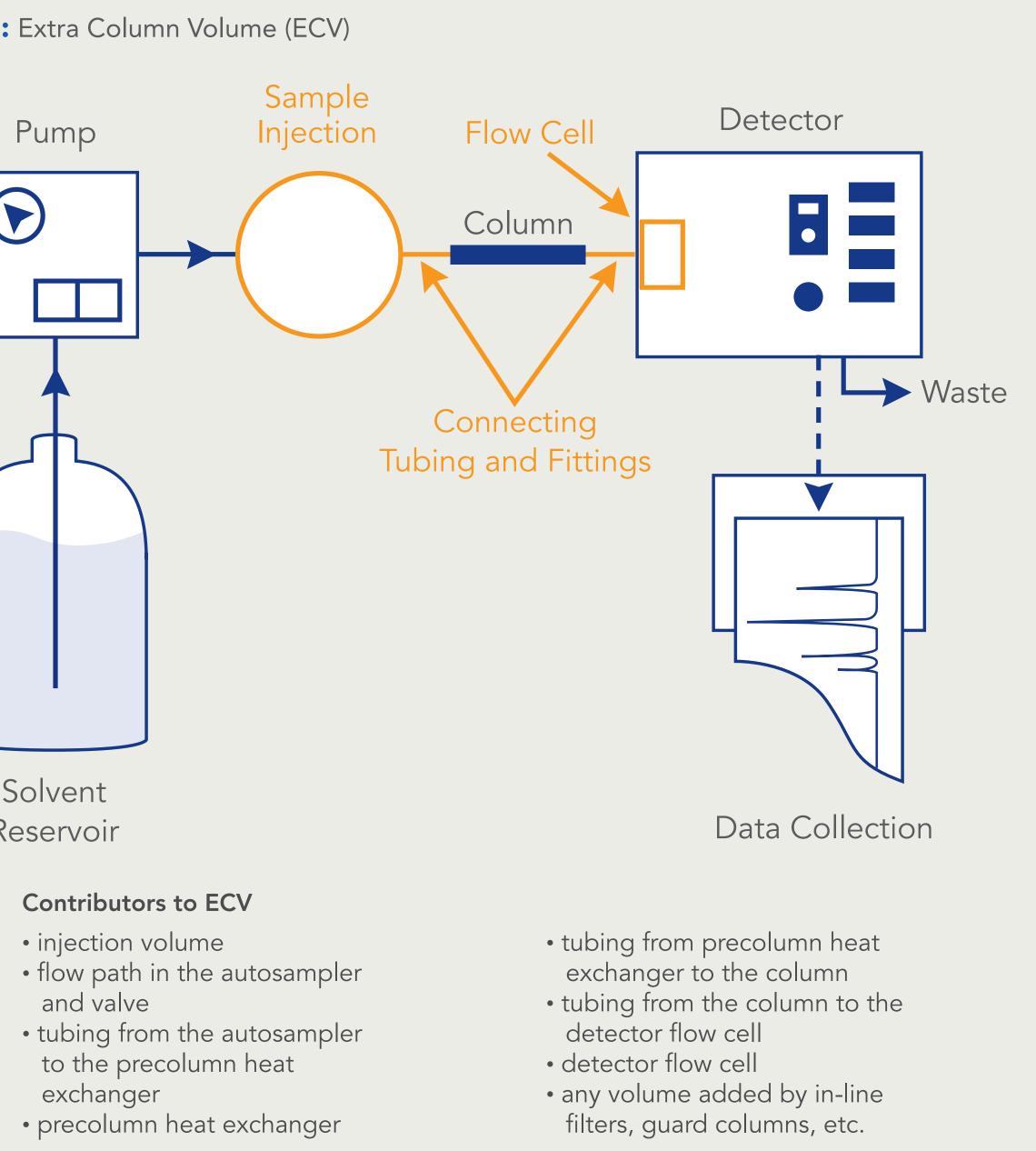


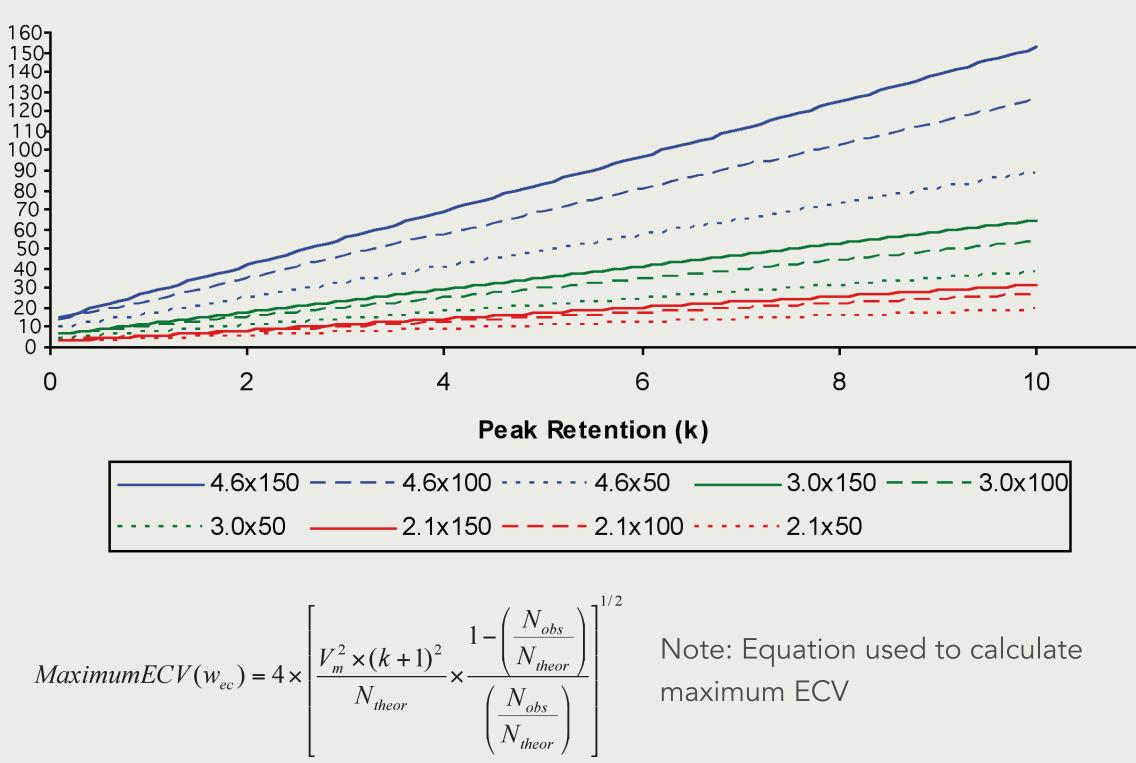
Overcoming Pressure Limitation

Conventional HPLC columns packed with 3 µm and 5 µm particles exhibit highest efficiency at mobile phase linear velocities between 0.13 and 0.23 cm/sec (Figure 1-A) and are generally operated in the range of 0.15 to 0.25 cm/sec., well within the pressure limit of conventional HPLC equipment (Figure 1-B). On the other hand, UHPLC columns yield higher efficiency at much faster linear velocities and it is typical that these columns will be operated in the linear velocity range of 0.3 to 0.5 cm/sec. Even a short 50 mm length UHPLC column packed with sub-2 µm particles will quickly exceed the maximum comfortable operating pressure of conventional HPLC equipment. UHPLC columns packed with Fused-Core particles are also typically run at flow velocities similar to other UHPLC columns, but their modest back pressure will not exceed the maximum operating pressure of conventional HPLC equipment, unless mobile phase linear velocity exceeds ~0.7 cm/sec.

Overcoming Excessive Extra Column Volume







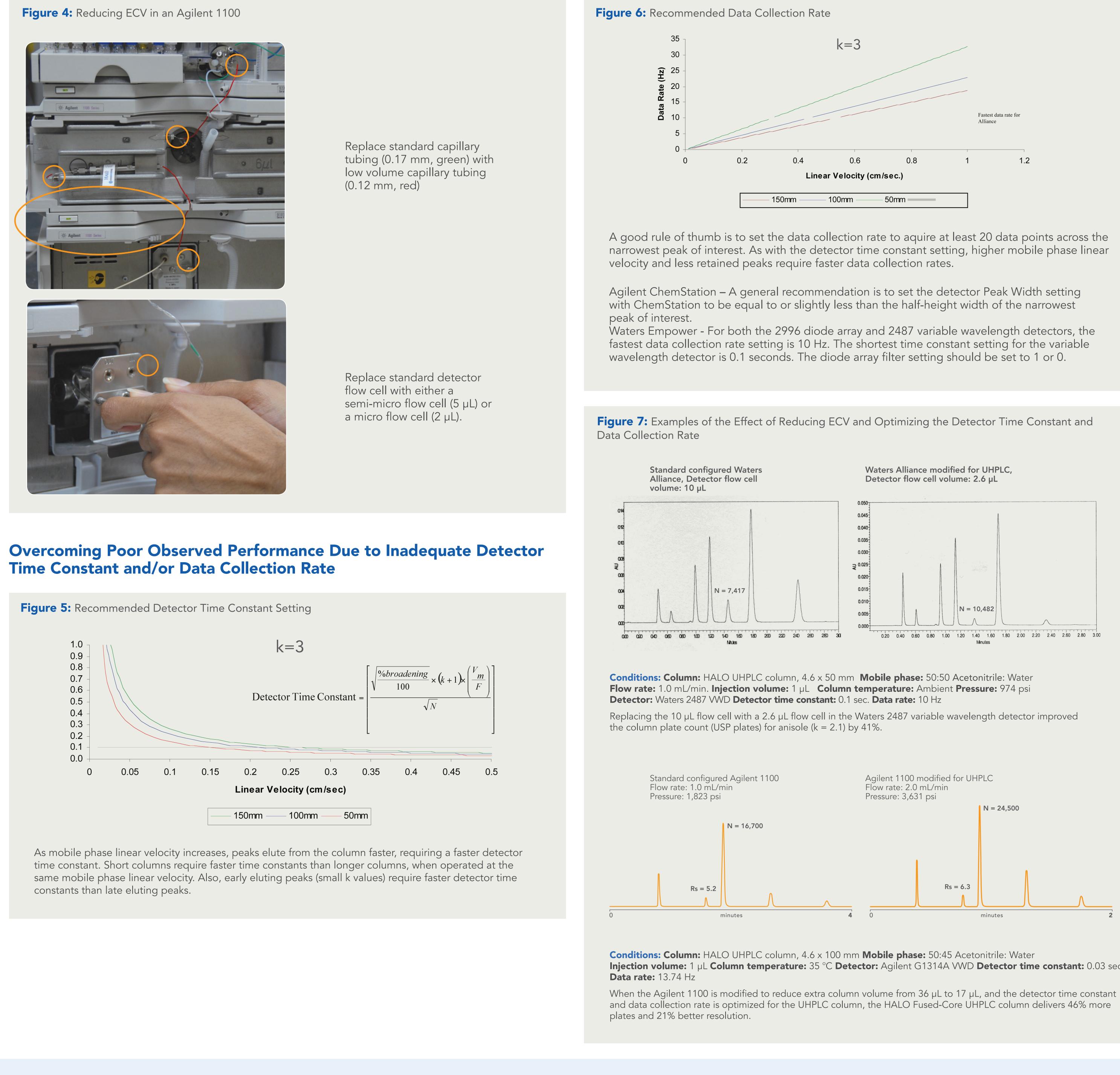
How to Achieve UHPLC-like Performance with Conventional HPLC Equipment

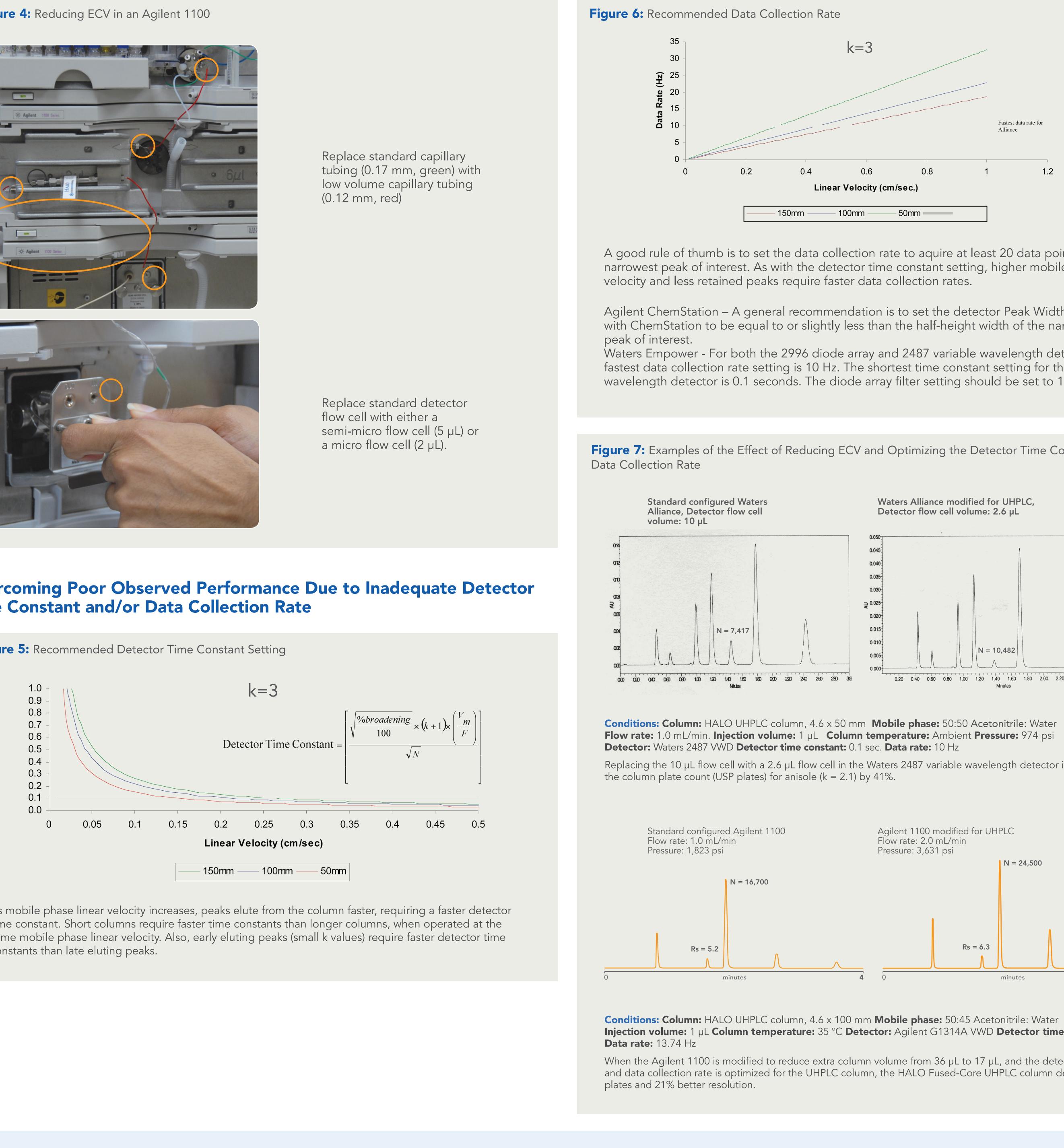
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to Maintain UHPLC Column Efficiency at >80% of Achievable

Fused-Core UHPLC columns, due to their much higher efficiency, generate peak volumes 20% to 40% smaller than conventional columns of the same dimensions packed with 3 µm to 5 µm particles. These smaller peak volumes place greater restrictions on system ECV. The smaller the column (length and ID) and the shorter the retention time of the analytes (k value), the smaller the equipment ECV has to be to achieve the resolving power possible from UHPLC columns. Figure 3 illustrates the maximum recommended ECV for different UHPLC column dimensions and different peak retention values (k) to achieve over 80% of the column efficiency and 90% of resolving power.





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Practical Limits of Modifying Conventional HPLC Equipment for Use with HALO Fused-Core UHPLC Columns

Depending on how a particular brand of HPLC is designed, there will be some limits to how much ECV can be eliminated from the system and further limits to the speed of the data collection rate and detector time constant. However, much can be done to improve the performance of most conventional HPLC systems when using UHPLC columns. We recommend the following steps:

- 1. Decrease the detector response time and increase the data collection rate. A good place to begin is to set the detector response time to < 0.3 seconds and the data collection rate to ≥ 10 Hz. Adjust the settings until an acceptable compromise between system efficiency and signal-to-noise is achieved.
- 2. Reduce sample injection volume to the minimum amount necessary to achieve acceptable peak response.
- **3.** Keep the sample solvent weaker than the mobile phase.
- 4. Replace the capillary connector tubing with smaller volume tubing of shorter lengths.
- 5. Replace the detector flow cell with a smaller volume flow cell.

Recommended equipment ECV, data rate, and time constant for maintaining optimum resolving power of Fused-Core UHPLC columns

Colui I.D. (mm)	Lengt	h Maximur		d Maximu or Data Ra	
4.6	100	33	15 µl	5	0.22
4.6	75	28	15 µl	10	0.19
4.6	50	23	15 µl	10	0.16
4.6	30	18	5 µl	13	0.12
3.0	100	14	5 µl	5	0.22
3.0	75	12	2 µl	10	0.19
3.0	50	10	2 µl	10	0.16
3.0	30	8	2 µl	13	0.12
2.1	100	7	2 µl	5	<0.1
2.1	75	6	2 µl	10	<0.1
2.1	50	5	1 µl	10	<0.1
2.1	30	4	1 µl	13	<0.1

Conclusions

UHPLC columns packed with Fused-Core particles (HALO) can be effectively used with conventional HPLC equipment if excessive extra column volume is removed and detector time constant and data collection rate settings are optimized for the particular Fused-Core column being used. Although these columns will yield the best performance when used with UHPLC systems, over 90% of their resolving power can be accessed when used with conventional HPLC equipment, if column dimensions, ECV, detector time constant and data collection rate are optimized. By taking the time to optimize these parameters, "UHPLC-like" performance can be achieved with most any conventional HPLC equipment.

