How to Use Conventional HPLC Equipment to Achieve UHPLC Performance

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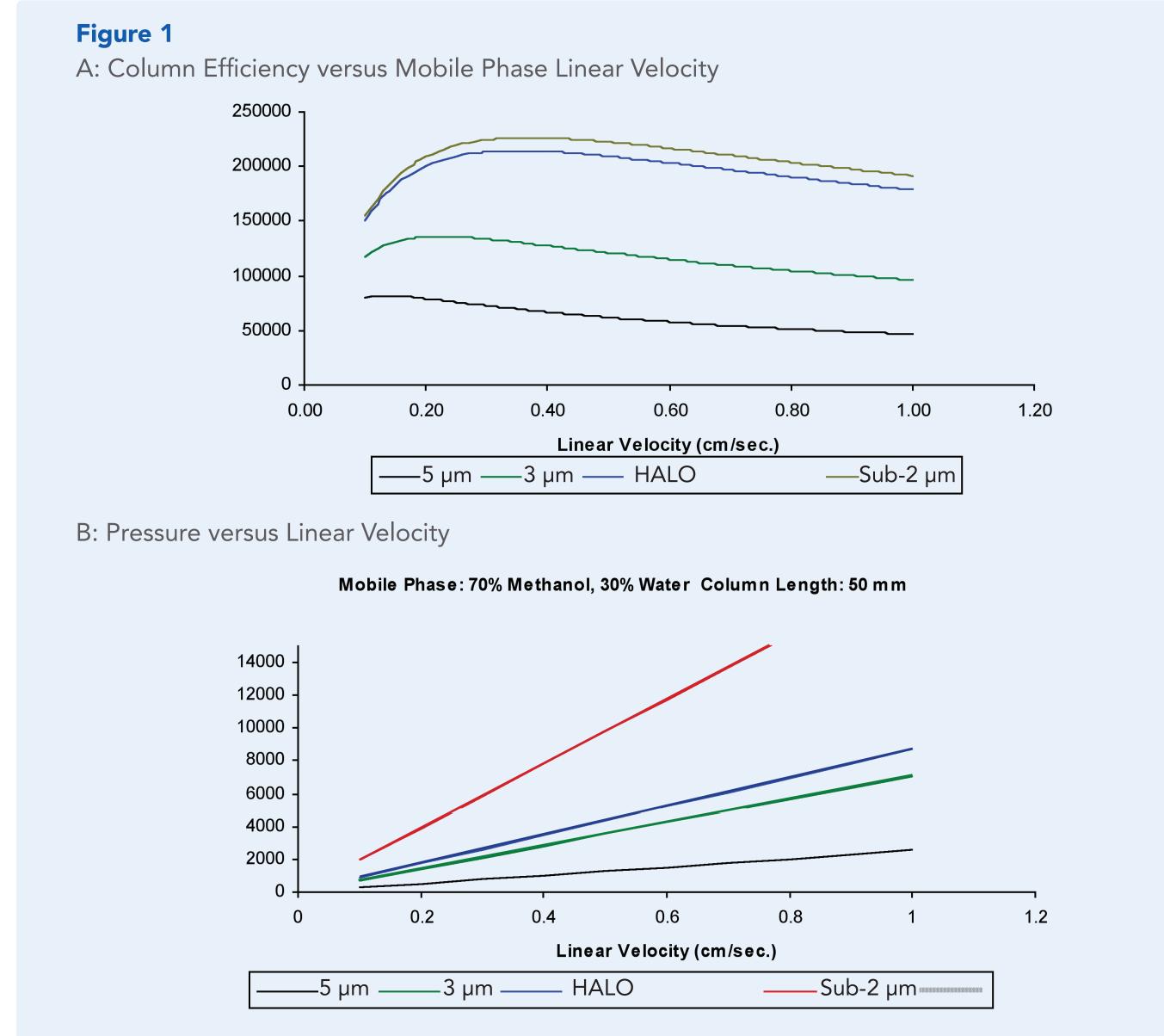
Introduction

In recent years the use of UHPLC has been exploited to significantly reduce chromatographic separation time compared to conventional HPLC. This high speed advantage has proved to be very useful, not only in improving productivity by reducing method development time, but also by increasing sample throughput. Nevertheless, new UHPLC equipment is expensive, which has deterred some laboratories from utilizing this technique. Fortunately, the introduction of new types of UHPLC columns, based on the use of sub-3-µm superficially porous particles, now offers the opportunity to achieve UHPLC performance using conventional equipment. Current commercially available UHPLC columns, such as HALO', packed with superficially porous particles operate effectively within the pressure limits of most conventional HPLCs. However, in order to achieve UHPLC performance with such columns, 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 chosen column dimensions and operating conditions. In this paper we will identify conventional HPLC instrument parameters that limit the performance of UHPLC columns and offer recommendations for modifying conventional HPLC equipment and adjusting method parameters to achieve UHPLC performance with columns packed with these new superficially porous particles.

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 HALO 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

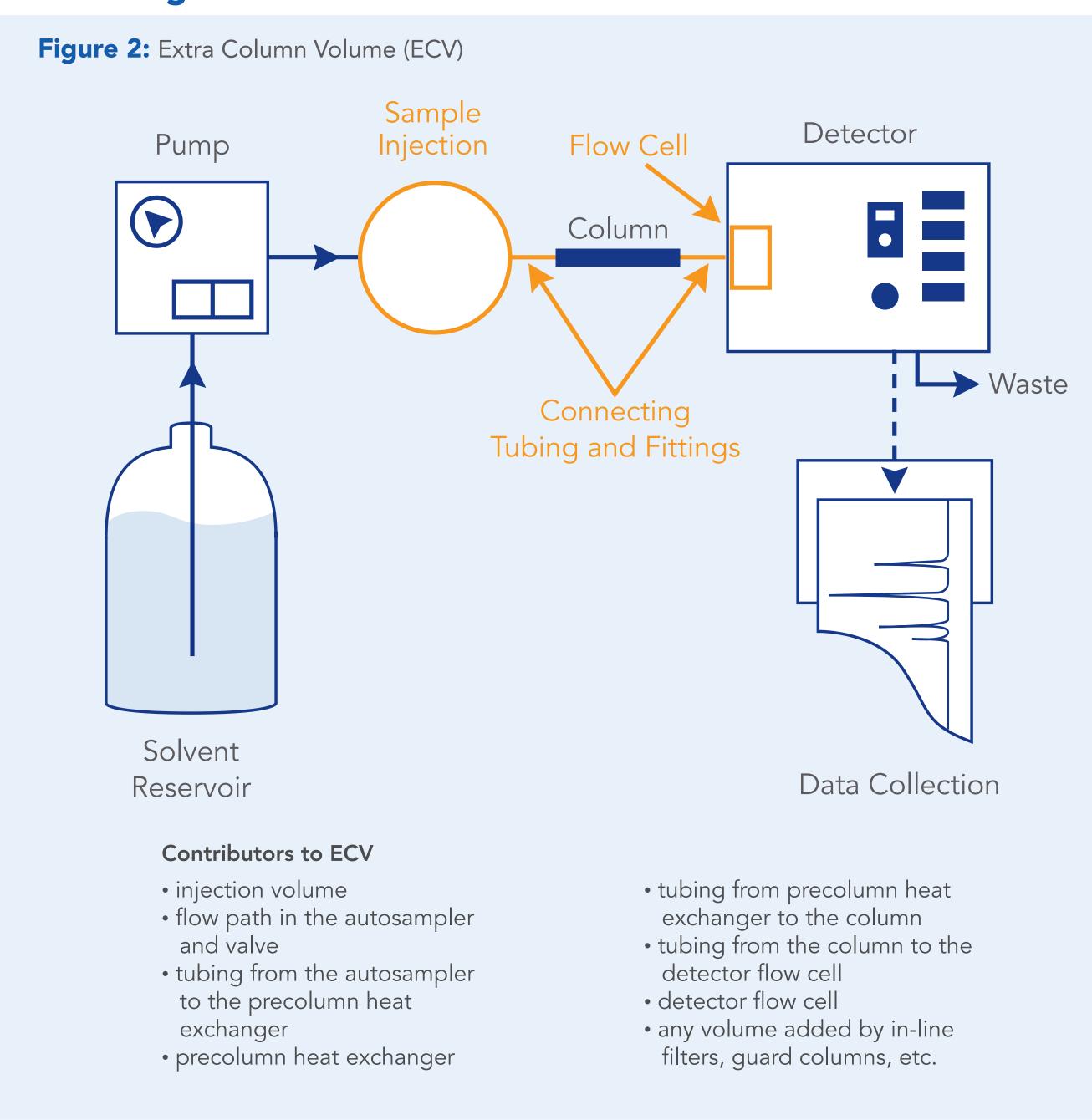
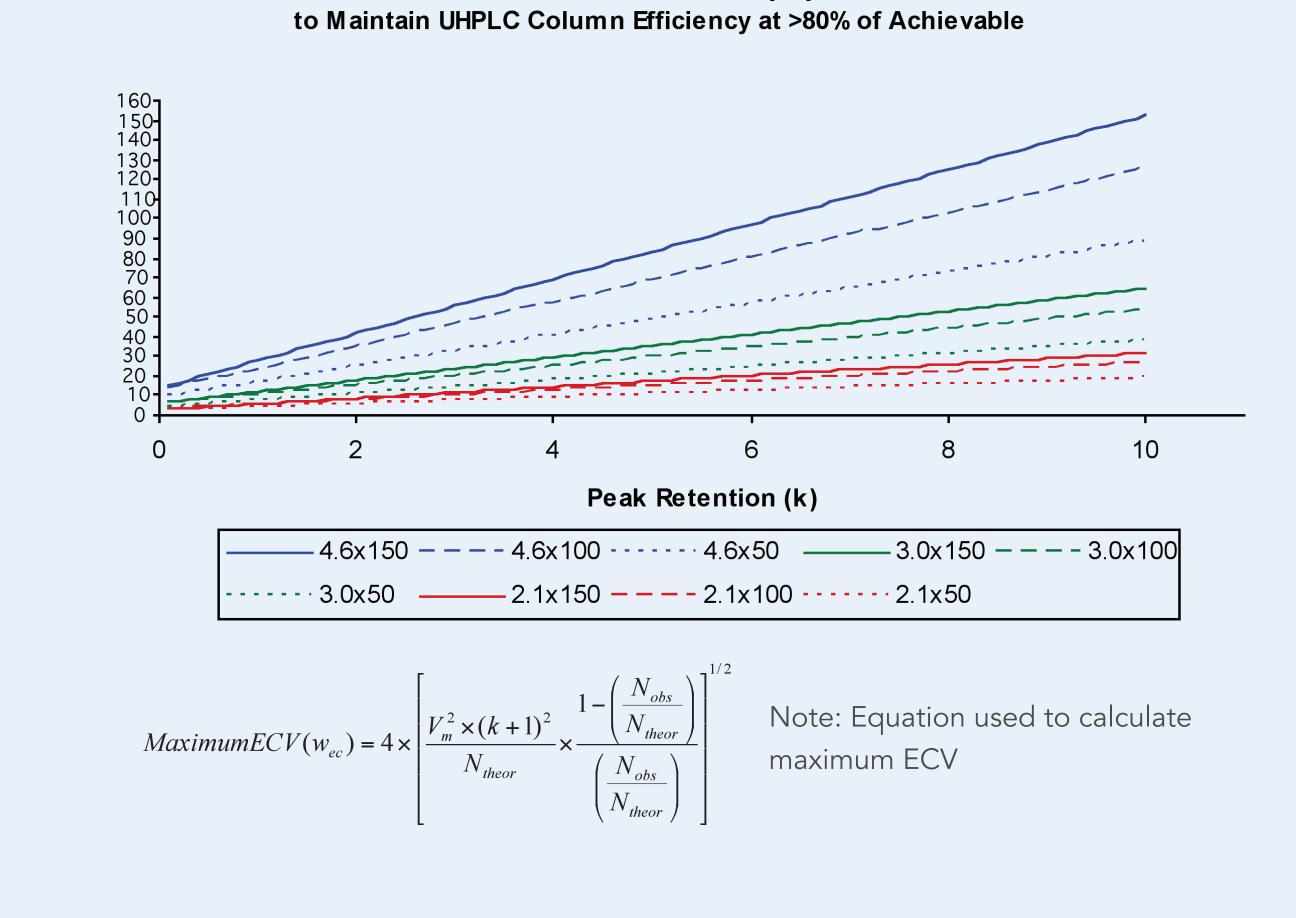
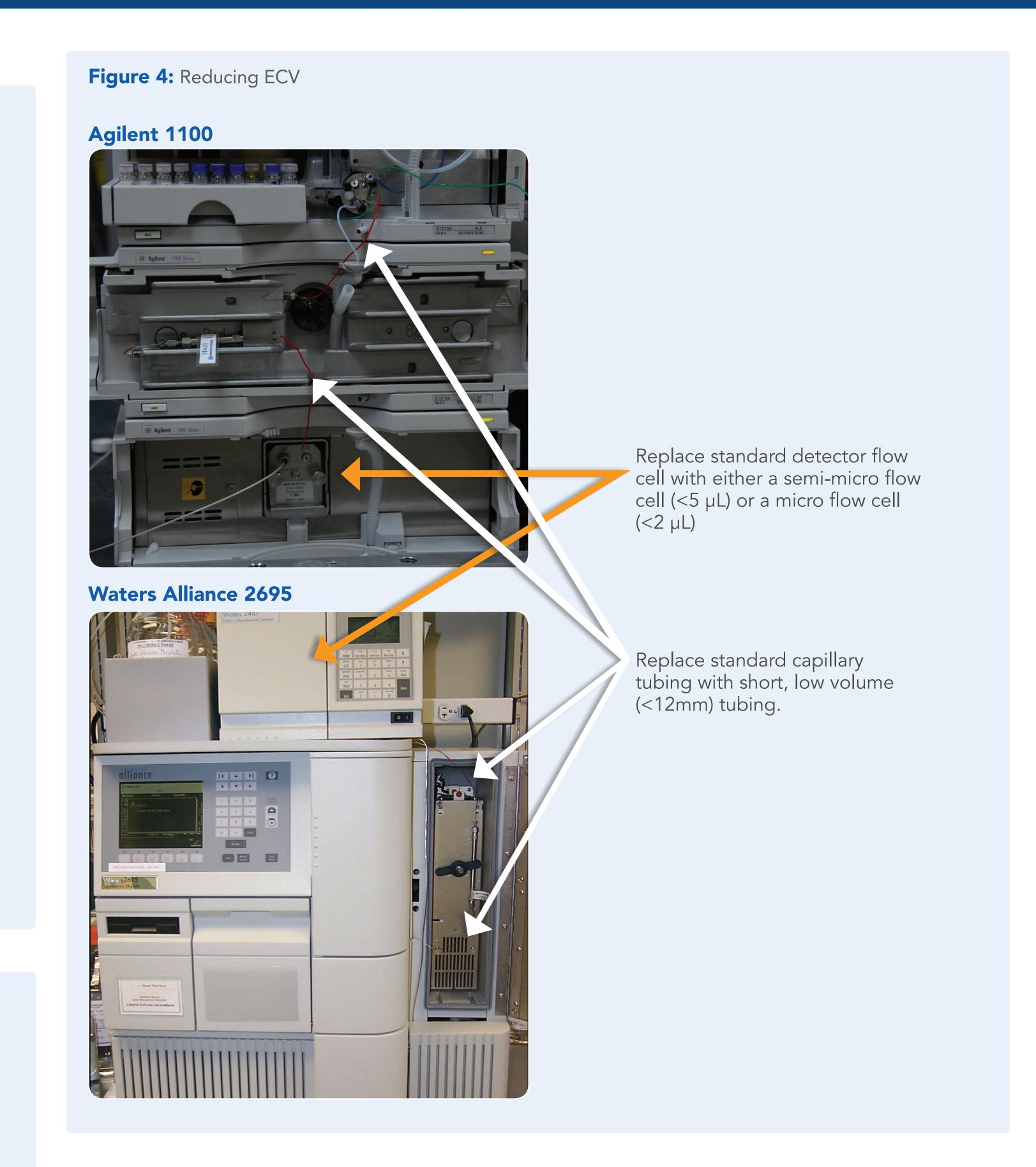


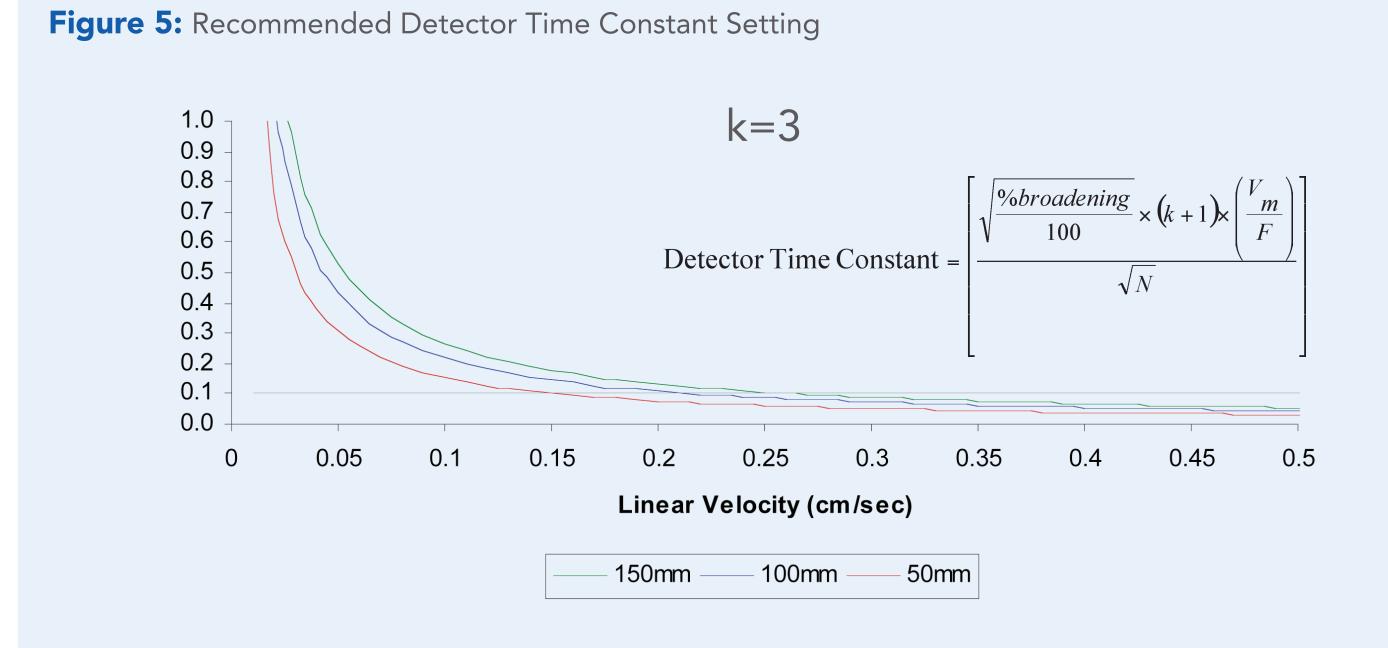
Figure 3: Maximum Recommended Equipment ECV



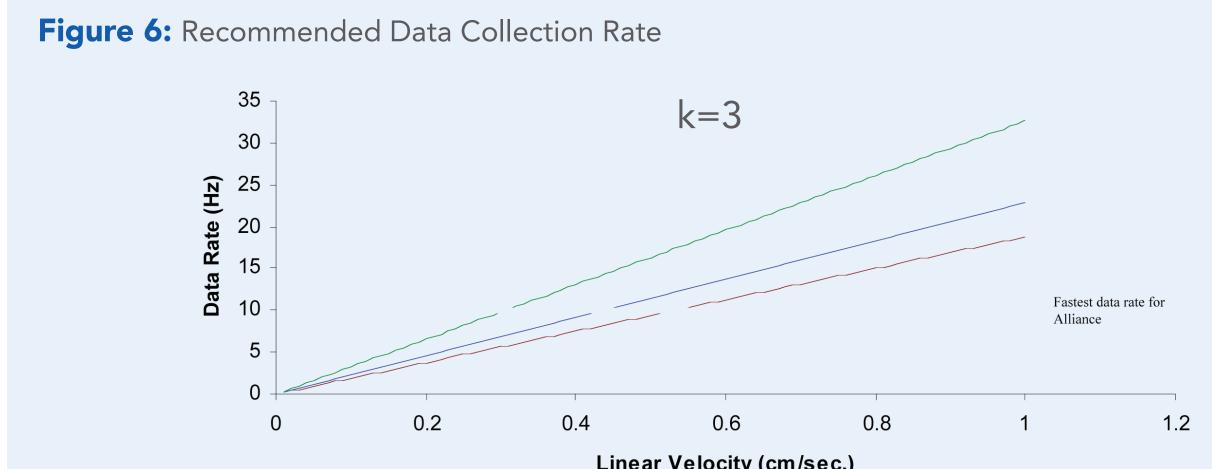
HALO UHPLC columns, due to their much higher efficiency, generate peak volumes 20% to 40% smaller than conventional columns of the same dimensions packed with 3 µm to 5 µm particles. These smaller peak volumes place greater restrictions on system ECV. The smaller the column (length and ID) and the shorter 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.



Overcoming Poor Observed Performance Due to Inadequate Detector Time Constant and/or Data Collection Rate



As mobile phase linear velocity increases, peaks elute from the column faster, requiring a faster detector time constant. Short columns require faster time constants than longer columns, when operated at the same mobile phase linear velocity. Also, early eluting peaks (small k values) require faster detector time constants than late eluting peaks.

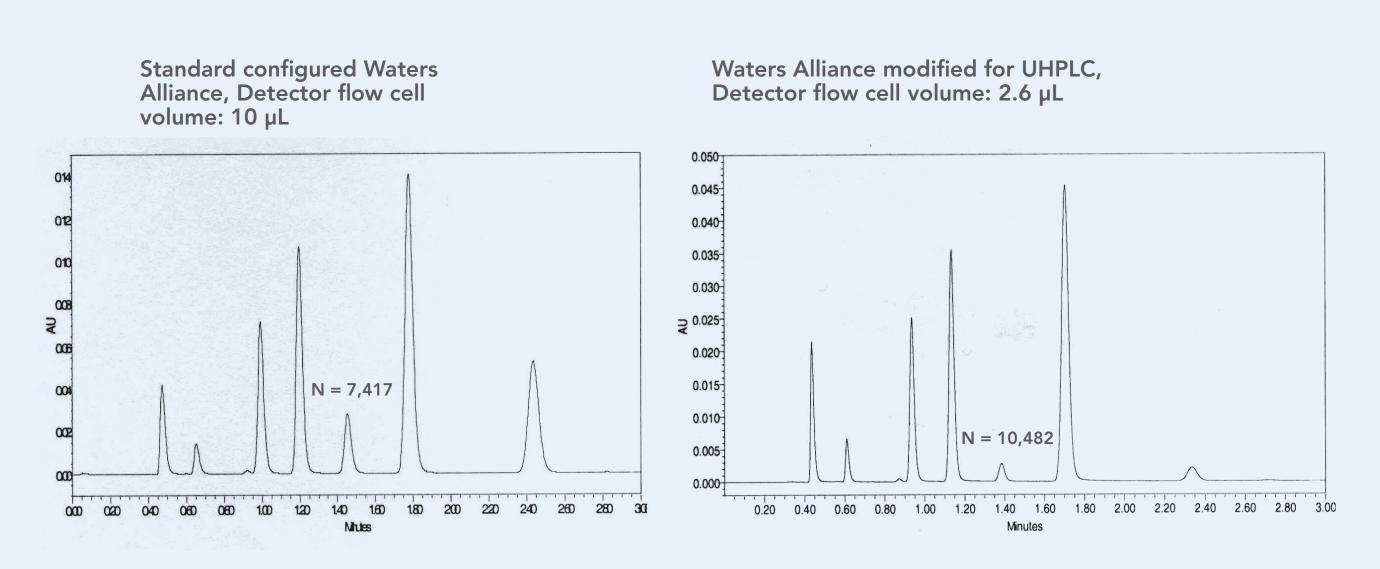


A good rule of thumb is to set the data collection rate to aquire at least 20 data points across the narrowest peak of interest. As with the detector time constant setting, higher mobile phase linear velocity and less retained peaks require faster data collection rates.

Agilent ChemStation – A general recommendation is to set the detector Peak Width setting with ChemStation to be equal to or slightly less than the half-height width of the narrowest peak of interest.

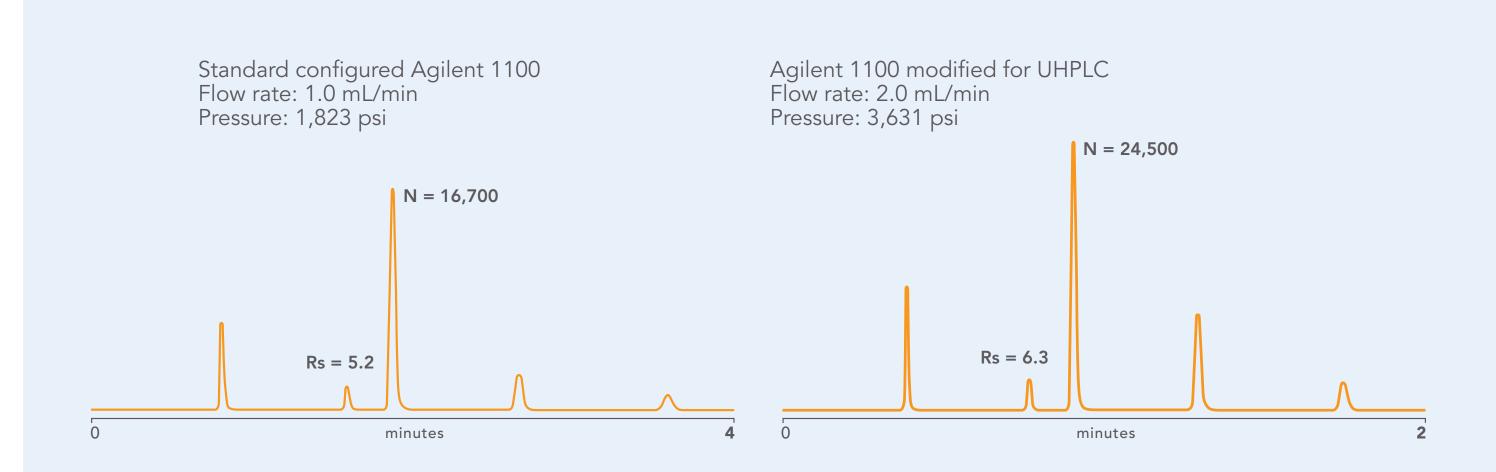
Waters Empower - For both the 2996 diode array and 2487 variable wavelength detectors, the fastest data collection rate setting is 10 Hz. The shortest time constant setting for the variable wavelength detector is 0.1 seconds. The diode array filter setting should be set to 1 or 0.

Figure 7: Examples of the Effect of Reducing ECV and Optimizing the Detector Time Constant and Data Collection Rate



Conditions: Column: HALO UHPLC column, 4.6 x 50 mm Mobile phase: 50:50 Acetonitrile: Water Flow rate: 1.0 mL/min. Injection volume: 1 µL Column temperature: Ambient Pressure: 974 psi Detector: Waters 2487 VWD Detector time constant: 0.1 sec. Data rate: 10 Hz

Replacing the 10 μ L flow cell with a 2.6 μ L flow cell in the Waters 2487 variable wavelength detector improved the column plate count (USP plates) for anisole (k = 2.1) by 41%.



Conditions: Column: HALO UHPLC column, 4.6 x 100 mm Mobile phase: 50:45 Acetonitrile: Water Injection volume: 1 µL Column temperature: 35 °C Detector: Agilent G1314A VWD Detector time constant: 0.03 sec.

Data rate: 13.74 Hz

When the Agilent 1100 is modified to reduce extra column volume from 36 μL to 17 μL, and the detector time constant and data collection rate is optimized for the UHPLC column, the HALO Fused-Core UHPLC column delivers 46% more plates and 21% better resolution.

Practical Limits of Modifying Conventional HPLC Equipment for Use with HALO 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 Paduca cample injection volume to the minimum amount page 200 minimu
- 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 UHPLC columns

Column .D. (mm)	Column Length (mm)	Total Maximum ECV (µl)	Maximum Volume Contributed by Detector Flow Cell	Maximum Data Rate (Hz)	Maximum Time Constant (sec)
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 μΙ	13	0.12
3.0	100	14	5 μΙ	5	0.22
3.0	75	12	2 μΙ	10	0.19
3.0	50	10	2 μΙ	10	0.16
3.0	30	8	2 μΙ	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

A new type of UHPLC column packed with sub-3-µm superficially porous particles, such as HALO°, can be effectively used with conventional HPLC equipment if excessive extra column volume is removed from the HPLC system and detector time constant and data rate collection settings are optimized for the specific UHPLC column being used. Although these sub-3-µm superficially porous UHPLC columns, as with other UHPLC columns, will yield the best performance with UHPLC equipment, over 90% of their resolving power can still be accessed when used with most conventional HPLC equipment, if time is taken to minimize extra column dispersion.