Modifying Conventional HPLC Equipment to Achieve UHPLC-like Performance with Fused-Core UHPLC Columns

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Introduction

The term "UHPLC" has generally been defined as "ultra high pressure liquid chromatography", with ultra high pressure described as pressure exceeding 400 bar (6,000 psi). However, the primary perceived benefit of UHPLC is the achievement of very fast separations with acceptable resolution of analytes, or resolution of very difficult-to-separate components. Until recently, it has not been possible to obtain UHPLC performance with conventional instruments because their excessive extra column volume and pressure limitations precluded fully utilizing existing UHPLC columns.

The introduction of a new type of UHPLC column based on Fused-Core particle technology (HALO) now offers the opportunity to achieve UHPLC performance with conventional equipment, because columns packed with Fused-Core particles operate effectively within the pressure limits of most conventional HPLCs. In order to deliver UHPLC performance, however, conventional instrumentation 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 dimension used.

This poster will identify the instrument parameters that can limit the performance of HALO Fused-Core columns with conventional HPLC systems, and will offer recommendations for modifying conventional HPLC equipment and method parameters to achieve UHPLC performance.

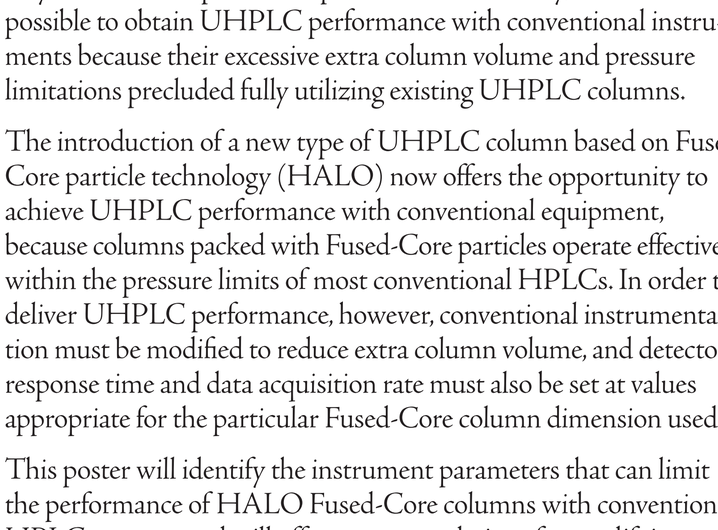
Three barriers to achieving UHPLC performance with conventional HPLC equipment

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

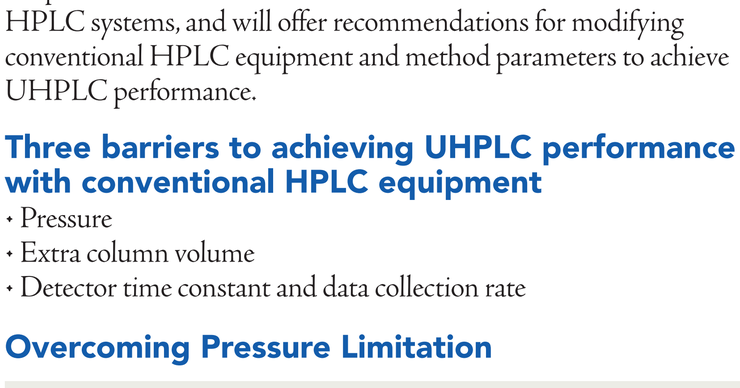
Overcoming Pressure Limitation

Figure 1

A: Column Efficiency versus Mobile Phase Linear Velocity



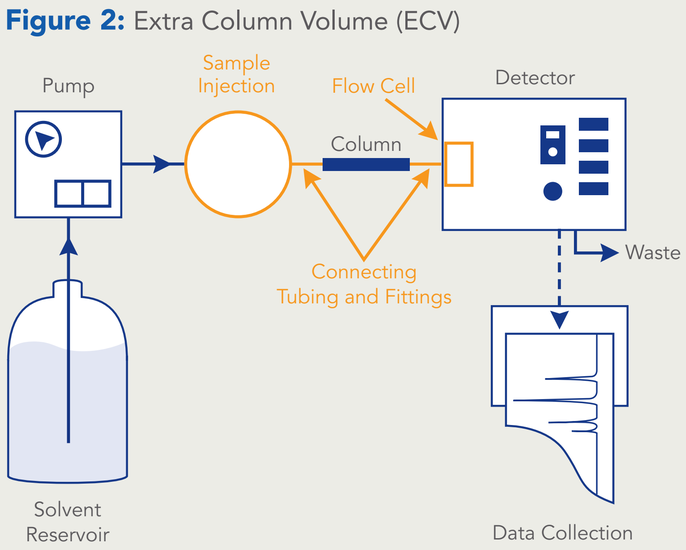
B: Pressure versus Linear Velocity



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

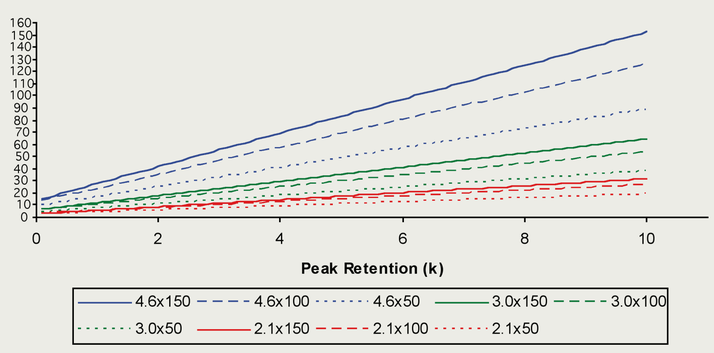
Figure 2: Extra Column Volume (ECV)



Contributors to ECV

- injection volume
- flow path in the autosampler and valve
- tubing from the autosampler to the precolumn heat exchanger
- precolumn heat exchanger
- tubing from precolumn heat exchanger to the column
- tubing from the column to the detector flow cell
- detector flow cell
- any volume added by in-line filters, guard columns, etc.

Figure 3: Maximum Recommended Equipment ECV to Maintain UHPLC Column Efficiency at >80% of Achievable



$$MaximumECV(w_{ec}) = 4 \times \left[\frac{V_m^2 \times (k+1)^2}{N_{theor}} \times \frac{1 - \left(\frac{N_{obs}}{N_{theor}}\right)}{\left(\frac{N_{obs}}{N_{theor}}\right)} \right]^{1/2}$$

Note: Equation used to calculate maximum ECV

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.

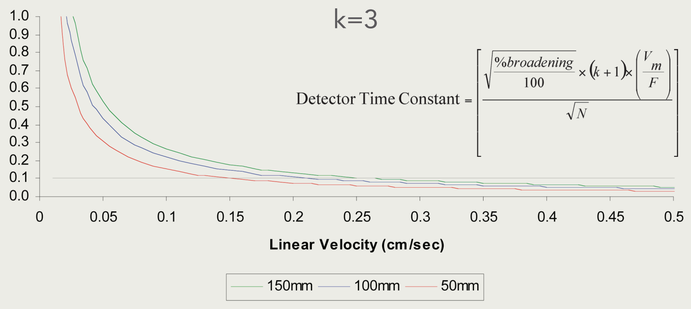
Figure 4: Reducing ECV in an Agilent 1100

Replace standard capillary tubing (0.17 mm, green) with low volume capillary tubing (0.12 mm, red)

Replace standard detector flow cell with either a semi-micro flow cell (5 µL) or a micro flow cell (2 µL).

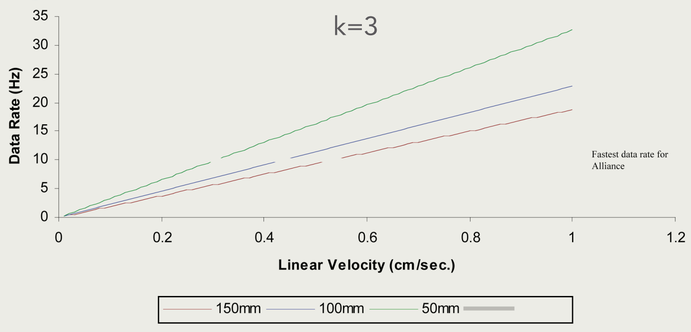
Overcoming Poor Observed Performance Due to Inadequate Detector Time Constant and/or Data Collection Rate

Figure 5: Recommended Detector Time Constant Setting



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.

Figure 6: Recommended Data Collection Rate



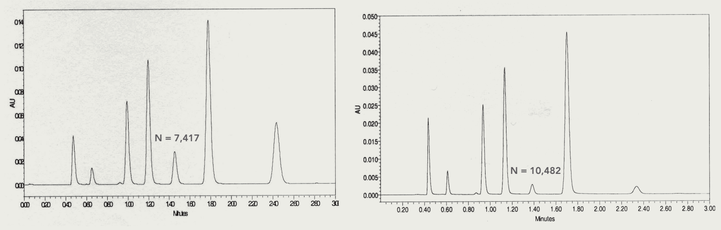
A good rule of thumb is to set the data collection rate to aquire at least 20 data points across the narrowest peak of interest. As with the detector time constant setting, higher mobile phase linear velocity and less retained peaks require faster data collection rates.

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

Standard configured Waters Alliance, Detector flow cell volume: 10 µL

Waters Alliance modified for UHPLC, Detector flow cell volume: 2.6 µL

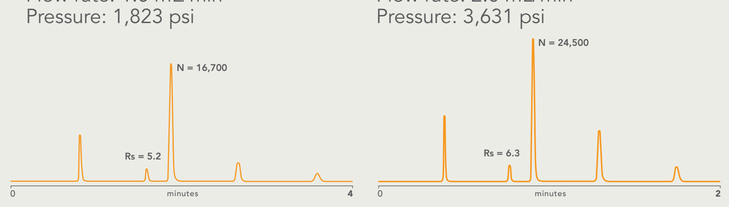


Conditions: Column: HALO UHPLC column, 4.6 x 50 mm **Mobile phase:** 50:50 Acetonitrile: Water **Flow rate:** 1.0 mL/min. **Injection volume:** 1 µL **Column temperature:** Ambient **Pressure:** 974 psi **Detector:** Waters 2487 VWD **Detector time constant:** 0.1 sec. **Data rate:** 10 Hz

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

Standard configured Agilent 1100
Flow rate: 1.0 mL/min
Pressure: 1,823 psi

Agilent 1100 modified for UHPLC
Flow rate: 2.0 mL/min
Pressure: 3,631 psi



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 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

Column I.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 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.

References
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