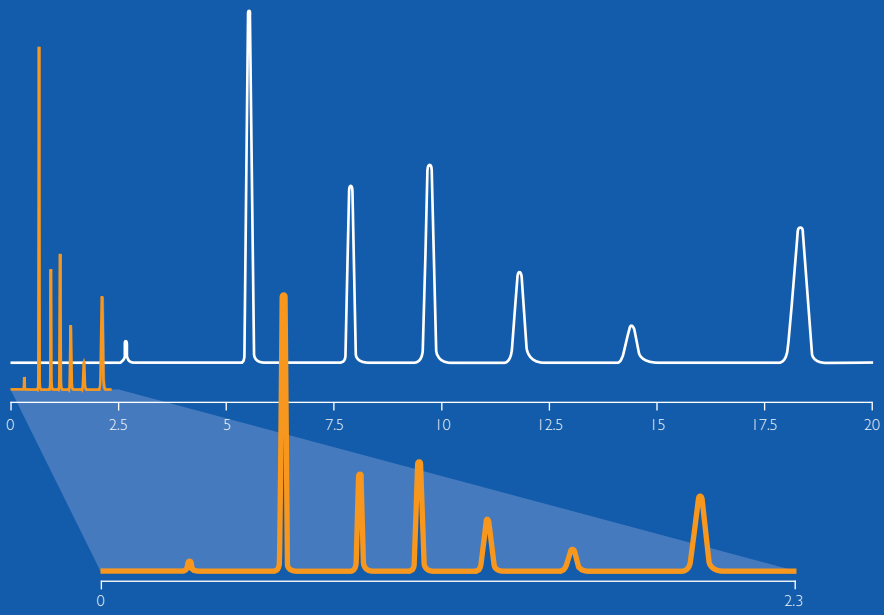
Quick Tips for Converting Conventional Reversed-Phase HPLC Separations to Ultra-Fast Separations



GUIDE TO ULTRA-FAST HPLC

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There has been enormous interest recently in so-called "ultra-fast" HPLC columns that can reduce run times by 70% or more. These ultra-fast columns are typically packed with particles considerably smaller than what is packed in conventional columns, giving them the advantage of equivalent separating power in much shorter length columns as well as the advantage that they maintain their separating power at higher mobile phase flow rates. The ability to use shorter columns and higher flow rates offers an opportunity to reduce analysis time and increase sample throughput significantly by substituting an ultra-fast column for a conventional column in an established method.

To facilitate converting conventional reversed-phase separations to ultra-fast separations, we have created this quick tips guide. It is intended to assist you in selecting an ultra-fast column and modifying conditions for a faster run time. In addition, this guide will help you estimate how the new ultra-fast conditions will affect run time, resolution, and back pressure.

Because peaks elute from ultra-fast columns faster and with much smaller volumes than conventional columns, modifications may have to be made to conventional HPLC equipment to obtain the full benefits that these columns offer. This Guide includes a section that offers hints on how to determine if a conventional LC will need to be modified for the ultra-fast column selected and offers advice on the modifications that should be made.

First, one qualification: An important chromatographic parameter that seems to be ignored in most discussions of ultra-fast HPLC is selectivity. Although selectivity is beyond the scope of this guide, you should be aware that converting from a conventional column to an ultra-fast column will sometimes be accompanied by a change in selectivity for one or more peak pairs in your chromatogram. This may be true if you continue to use the same bonded phase chemistry, such as C18, and even true if the ultra-fast column you choose is the same brand as the conventional column that it is replacing. However, in most cases the change in selectivity will be minor enough that the estimation models used in this guide will still be useful.

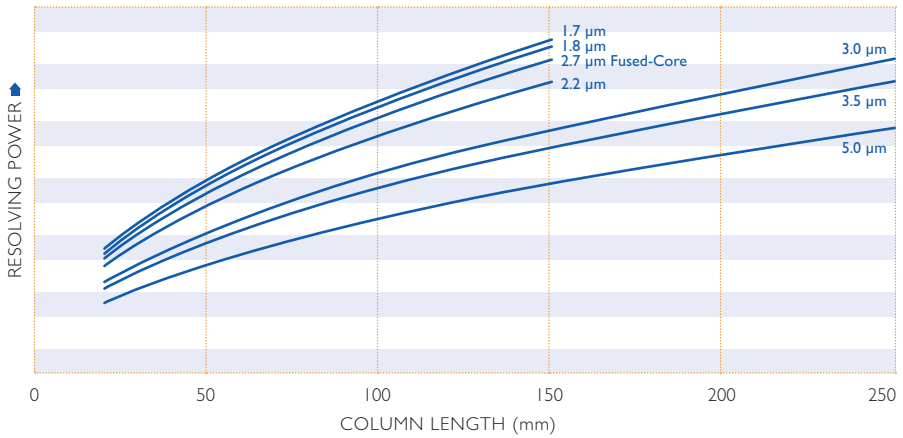
Suggested Steps for Converting a Conventional Reversed-Phase Separation to an Ultra-Fast Separation

1. Select the shortest ultra-fast column that can provide resolution equivalent to or better than the conventional column. *See Figure A.*

2. Estimate the back pressure for the selected "ultra-fast" column. *See Figure B.* If the pressure exceeds the maximum acceptable pressure for your system, select an alternate column with lower back pressure, most likely one packed with larger particles. You could elect to operate at a lower flow rate to keep the pressure acceptable, but this would also increase the run time, negating the purpose of converting to an ultra-fast column.

Figure A: Resolving Power as a Function of Particle Size and Column Length

Instructions: This chart plots resolving power (the ability of a column to separate components in a mixture) versus column length for 7 different columns. Three of the columns are packed with conventional particles (5.0 µm, 3.5 µm and 3.0 µm) and four are packed with ultra-fast particles (2.2 µm, 2.7 µm Fused-Core®, 1.8 µm and 1.7 µm). As column length increases, so does resolving power, but run time also increases. Notice that the ultra-fast columns provide greater resolving power in much shorter column lengths compared to the conventional columns. When converting a conventional separation to an ultra-fast separation, choose the shortest ultra-fast column that provides resolving power equal to or better than the conventional column it is replacing. This will allow you to minimize run time and maintain acceptable resolution.



Example: A 100 mm ultra-fast column packed with 2.7 µm Fused-Core particles meets the criteria of providing equal or better resolving power compared to a 250 mm column packed with conventional 5 µm particles. This ultra-fast column is an appropriate choice for replacing the 250 mm length conventional column in an ultra-fast method.

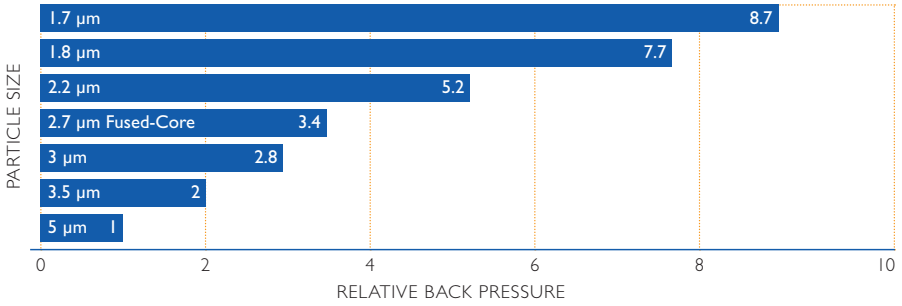
Figure B: Relative Back Pressure versus Particle Size

Instructions: For the ultra-fast column configuration selected in Step 1 (length, particle size), estimate the expected back pressure on this column by multiplying the pressure observed on the conventional column by the ratio of the "Relative Pressure" of the ultra-fast column to the conventional column and then by the ratio of the column lengths.

Note: This calculation assumes that the mobile phase velocity is the same for both the conventional column and the ultra-fast column.

$$P_2 = P_1 \times \frac{RP_2}{RP_1} \times \frac{L_2}{L_1}$$

- P_2: Estimated back pressure of the ultra-fast column
- RP_2: Relative back pressure of the ultra-fast column
- L_2: Length of the ultra-fast column
- P_1: Measured back pressure of conventional column
- RP_1: Relative back pressure of the conventional column
- L_1: Length of the conventional column



Example: A 100 mm ultra-fast column packed with 1.8 µm particles will generate approximately 3 times the back pressure of a 250 mm conventional column packed with 5 µm particles.

$$P_{\text{ULTRA-FAST COLUMN}} = P_1 \times \frac{7.7}{1} \times \frac{100\text{ mm}}{250\text{ mm}} = 3.08 \times P_{\text{CONVENTIONAL COLUMN}}$$

3. Confirm that the selectivity and resolution of the ultra-fast column is adequate. Since the selectivity of the ultra-fast column may be different from the selectivity of the conventional column (this may be true even if both the conventional column and the ultra-fast column are the same brand), run your separation with the ultra-fast column and calculate resolution. If the resolution does not meet the minimum required resolution, you may have to choose a longer column, or possibly even a different brand of ultra-fast column (with a different selectivity) to achieve acceptable resolution. If the resolution exceeds the required resolution, you may be able to use an even shorter ultra-fast column, or at least operate at a higher mobile phase flow rate to reduce the run time even further.

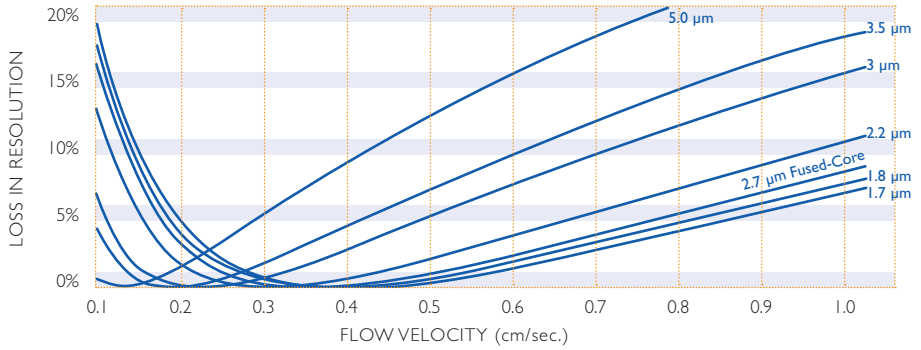
4. Once a column has been selected that provides acceptable resolution and pressure, increase the flow rate to minimize the run time while maintaining acceptable resolution and pressure. Considerable time savings and greater sample throughput can be achieved by operating at higher flow rates with an ultra-fast column, as long as you don't exceed your system's maximum back pressure. *See Figure C.*

Figure C: Resolution versus Mobile Phase Flow Rate

Instructions: If the resolution on the selected ultra-fast column exceeds the minimum required resolution for the separation and does not exceed the pressure limit, you will be able to reduce analysis time further by increasing the flow rate. Since the optimum flow velocity (for maximum resolution) of an ultra-fast column is 3 to 4 times faster than for a conventional column, you may actually be able to both reduce the run time and increase resolution by operating at a higher flow rate.

This chart estimates change in resolution with changes in mobile phase velocity. Not only do ultra-fast columns have their optimum efficiency at higher mobile phase velocities, they also sacrifice less of their efficiency as mobile phase velocity is increased beyond their optimum.

Change in Resolution with Flow Rate



Example: An ultra-fast column packed with 2.7 µm Fused-Core particles can be operated at a relatively fast mobile phase velocity of 0.7 cm/sec and still retain over 96% of its resolving power. A conventional column packed with 5 µm particles run at the same flow velocity would retain only about 82% of its resolving power.

Converting Mobile Phase Velocity (cm/sec) to Column Flow Rate (ml/min)

Mobile Phase Velocity	Column ID (mm)				Mobile Phase Velocity	Column ID (mm)			
	1.0	2.1	3.0	4.6		1.0	2.1	3.0	4.6
0.1	0.030	0.13	0.27	0.63	0.6	0.18	0.79	1.6	3.8
0.2	0.059	0.26	0.53	1.3	0.7	0.21	0.92	1.9	4.4
0.3	0.089	0.39	0.80	1.9	0.8	0.24	1.0	2.1	5.0
0.4	0.12	0.52	1.1	2.5	0.9	0.27	1.2	2.4	5.7
0.5	0.15	0.65	1.3	3.1	1.0	0.30	1.3	2.7	6.3

5. If the separation uses gradient elution, you will need to adjust the gradient time (t_G) to the volume of the ultra-fast column and for any changes in flow rate. *See Figure D.*

Figure D: Adjust Gradient Time (t_G) for Changes in Column Volume and Flow Rate

$$t_{G2} = t_{G1} \times \frac{V_{m2}}{V_{m1}} \times \frac{F_1}{F_2}$$

t_{G2}: Gradient time for the ultra-fast separation
V_{m2}: Column volume of the ultra-fast column (see Table 1)
F_2: Flow rate for the ultra-fast separation

t_{G1}: Gradient time for the conventional separation
V_{m1}: Column volume of the conventional column (see Table 1)
F_1: Flow rate for the conventional separation

Important Note: The system dwell volume (gradient mixing volume) can have a significant effect on the chromatography when using gradients because it adds an isocratic hold to the beginning of the gradient. The time of this "hold" is equal to the dwell volume divided by the flow rate. When the flow rate is changed, this isocratic hold will also change. This change in gradient hold will generally have more of an effect on early eluting peaks, but it will also affect all peaks in the chromatogram to some extent. To minimize the effect on your separation, keep the dwell volume as small as possible by using micro gradient mixers and keeping the tubing volume in the system to a minimum.

Example: A conventional method uses a column 4.6 x 150 mm (1.57 ml), a flow rate of 1.0 ml/min, and a gradient of 15% B to 35% B in 20.0 minutes. The gradient time for an ultra-fast method that uses a column 4.6 x 50 mm (2.7 µm Fused-Core, 0.42 ml) and a flow rate of 2.0 ml/min is:

$$t_{G\ ULTRA\text{-}FAST} = 20 \text{ minutes} \times \frac{0.42\text{ml}}{1.57\text{ml}} \times \frac{1.0\text{ml/min}}{2.0\text{ml/min}} = 2.7 \text{ min.}$$

See Table 1, page 11 for column volume (V_m) estimates.

6. Adjust the sample injection volume to the ultra-fast column's volume. *See Figure E.*

Figure E: Adjust the Sample Injection Volume for Changes in Column Dimension

$$S_{v2} = S_{v1} \times \frac{V_{m2}}{V_{m1}}$$

S_{v2}: Injected sample volume for the ultra-fast column

S_{v1}: Injected sample volume for the conventional column

V_{m2}: Volume of the ultra-fast column (see Table 1)

V_{m1}: Volume of the conventional column (see Table 1)

Example: A conventional method uses a sample injection volume of 20 µl on a column 4.6 x 150 mm.

The sample volume that should be injected on a 4.6 x 50 mm ultra-fast column (2.7 µm Fused-Core) is:

$$S_{v\ ULTRA\text{-}FAST} = 20\ \mu l \times \frac{0.42\ ml}{1.57\ ml} = 5\ \mu l$$

See Table 1, page 11 for column volume (V_m) estimates.

Example: Converting a Conventional Separation to an Ultra-Fast Separation.

Conventional HPLC Separation Conditions

COLUMN: 4.6 x 250 mm, 5 µm
FLOW RATE: 1.5 ml/min
MOBILE PHASE: Isocratic
RUN TIME: 10 minutes
PRESSURE: 1,580 psi, 109 bar
Maximum acceptable pressure = 4,000 psi, 275 bar
RESOLUTION: 3.0
SAMPLE INJECTION VOLUME: 20 µl

Converting to Ultra-Fast Separation Conditions

1. Select the shortest ultra-fast column that provides resolution equivalent to or better than the conventional column. ***(See Relative Resolution chart in Figure A.)***

A column 4.6 x 100 mm packed with 1.7 µm particles is selected for further investigation.

2. Estimate back pressure. ***(See relative pressure table in Figure B.)***

$$P_{\text{Ultra-Fast Column}} = 1{,}580 \text{ psi} \times \frac{8.7}{1} \times \frac{100 \text{ mm}}{250 \text{ mm}} = 5{,}498 \text{ psi}$$

Since this ultra-fast column exceeds our maximum acceptable back pressure (4,000 psi), a different ultra-fast column is selected for investigation.

The alternative ultra-fast column selected is a 4.6 x 100 mm packed with 2.7 µm Fused-Core particles (HALO® HPLC column). The back pressure on this column is:

$$P_{\text{Ultra-Fast Column}} = 1{,}580 \text{ psi} \times \frac{3.4}{1} \times \frac{100 \text{ mm}}{250 \text{ mm}} = 2{,}149 \text{ psi}$$

This ultra-fast column provides both acceptable resolution and acceptable back pressure for our method.

3. Confirm that the selectivity and resolution of the ultra-fast column is adequate.

For simplicity, we will assume that the selectivity of this ultra-fast column is almost identical to the selectivity of the conventional column and, therefore, the resolution is adequate.

4. Optimize flow rate to minimize run time. ***(See Figure C to estimate changes in resolution with changes in flow rate.)***

We can further reduce run time by operating the ultra-fast column at a higher flow rate. We just have to make sure we stay within the requirements of minimum resolution and maximum pressure. The ultra-fast column we selected has low enough back pressure that we can operate at a flow rate of 2.5 ml/min and still stay within our defined limits of pressure and resolution.

$$P_{\text{at 2.5 ml/min}} = 2{,}149 \text{ psi} \times \frac{2.5 \text{ ml/min}}{1.5 \text{ ml/min}} = 3{,}582 \text{ psi}$$

5. Adjust the gradient time.

This is an isocratic separation, so no adjustment to gradient time is required.

6. Adjust the sample injection volume. ***(See Figure D for calculations and table with estimated column volumes.)***

$$\text{Sample volume} = 20 \text{ µl} \times \frac{0.84 \text{ ml}}{2.62 \text{ ml}} = 6.4 \text{µl}$$

Ultra-Fast Conditions

COLUMN: 4.6 x 100 mm, 2.7 µm Fused-Core (HALO)
FLOW RATE: 2.5 ml/min
RUN TIME: $10 \text{ min} \times \frac{0.84 \text{ ml}}{2.62 \text{ ml}} \times \frac{1.5 \text{ ml/min}}{2.5 \text{ ml/min}} = 1.9 \text{ min}^*$
RESOLUTION: 3.1
PRESSURE: 3,582 psi
SAMPLE INJECTION VOLUME: 6.4 µl

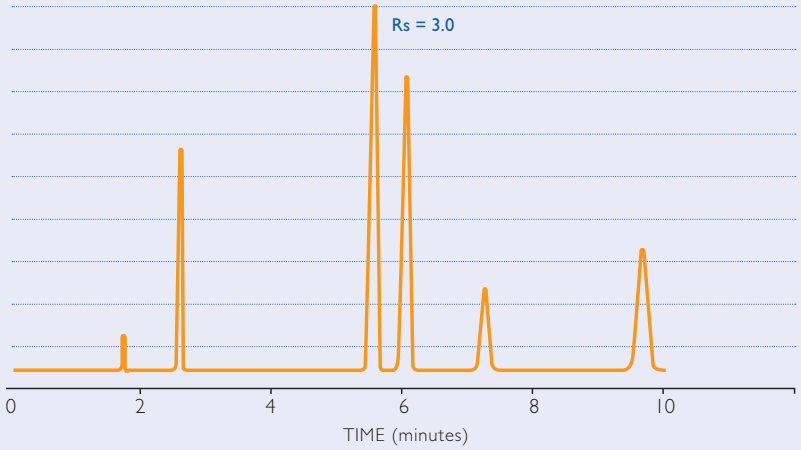
* Run time for the ultra-fast separation can be estimated by multiplying the run time on the conventional column by the ratio of the volumes of ultra-fast column to the conventional column and then by the inverse ratio of the flow rates on the two columns. (See page 11)

Figure F: Converting a conventional separation to an ultra-fast separation

Conventional Separation

Columns: 4.6 x 250 mm, 5 µm C18
Flowrate: 1.5 ml/min
Resolution: 3.0

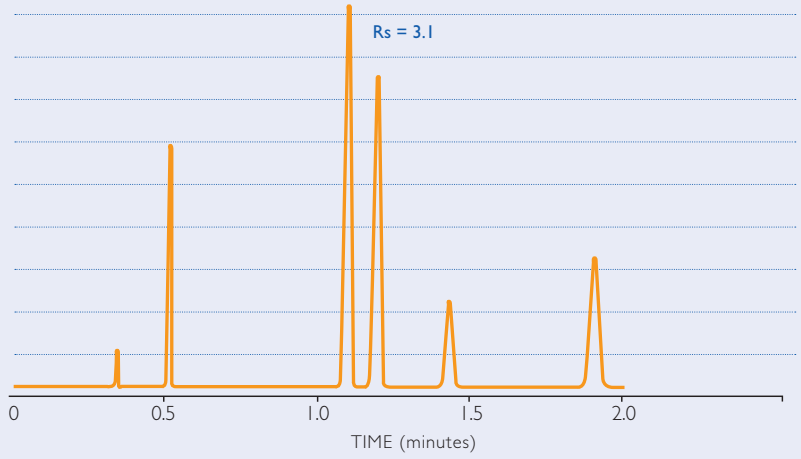
Pressure: 1,580 psi
Run Time: 10 minutes
Sample Injection Volumn: 20 µl



Ultra-Fast Separation

Columns: 4.6 x 100 mm, 2.7 µm Fused-Core C18 (HALO® HPLC column)
Flowrate: 2.5 ml/min
Resolution: 3.1

Pressure: 3,582 psi
Run Time: 1.9 minutes
Sample Injection Volumn: 6.4 µl



These are simulated chromatograms generated from theoretical data. Differences in selectivity between a conventional column and an ultra-fast column or changes in secondary retention interactions will yield different chromatographic results from what is predicted here.

Reference Tables and Equations for Quick Estimates

Table 1: Estimated volume, V_m, for a variety of available column dimensions

ID (mm)	Length (mm)	V_m (ml)	V_m Fused-Core (ml)	ID (mm)	Length (mm)	V_m (ml)	V_m Fused-Core (ml)
1.0	20	0.010	0.008	3.0	20	0.089	0.071
1.0	30	0.015	0.012	3.0	30	0.134	0.107
1.0	50	0.025	0.020	3.0	50	0.223	0.178
1.0	75	0.037	0.030	3.0	75	0.334	0.267
1.0	100	0.050	0.040	3.0	100	0.445	0.356
1.0	150	0.074	0.059	3.0	150	0.668	0.534
1.0	250	0.124	0.099	3.0	250	1.11	0.89
2.1	20	0.044	0.035	4.6	20	0.209	0.168
2.1	30	0.066	0.052	4.6	30	0.314	0.251
2.1	50	0.109	0.087	4.6	50	0.524	0.419
2.1	75	0.164	0.131	4.6	75	0.785	0.628
2.1	100	0.218	0.175	4.6	100	1.05	0.84
2.1	150	0.327	0.262	4.6	150	1.57	1.26
2.1	250	0.546	0.436	4.6	250	2.62	2.09

Note: Column volumes listed here are estimates only. However, most commercial columns can be expected to have volumes within about 5% of what is reported here.

Table 2: Column plate number, N, for columns packed with different size/type particles

Particle	N per cm of column length	Particle	N per cm of column length
5 µm	800	2.7 µm Fused-Core	2200
3.5 µm	1140	1.8 µm	2400
3 µm	1330	1.7 µm	2500
2.2 µm	1900		

Note: Estimates in this Table are for near ideal conditions. Column plate number is dependent on many factors including the solute, mobile phase viscosity and flow velocity and it is not unusual under "real-world" conditions for column plate numbers to be over 20% lower than what is reported here.

Table 3: Reference Equations

$$Rs_{ultra\text{-}fast} = Rs_{conventional} \times \sqrt{\frac{N_{ultra\text{-}fast}}{N_{conventional}}}$$

$$RT_{ultra\text{-}fast} = RT_{conventional} \times \frac{V_{ultra\text{-}fast}}{V_{conventional}} \times \frac{F_{conventional}}{F_{ultra\text{-}fast}}$$

$$t_{g\ ultra\text{-}fast} = t_{g\ conventional} \times \frac{V_{ultra\text{-}fast}}{V_{conventional}} \times \frac{F_{conventional}}{F_{ultra\text{-}fast}}$$

$$Sv_{ultra\text{-}fast} = Sv_{conventional} \times \frac{V_{ultra\text{-}fast}}{V_{conventional}}$$

Rs = Resolution
N = Column Plate Number
RT = Run Time
V = Column Volume
F = Flow Rate
Sv = Injected Sample Volume
t_g = Gradient Time

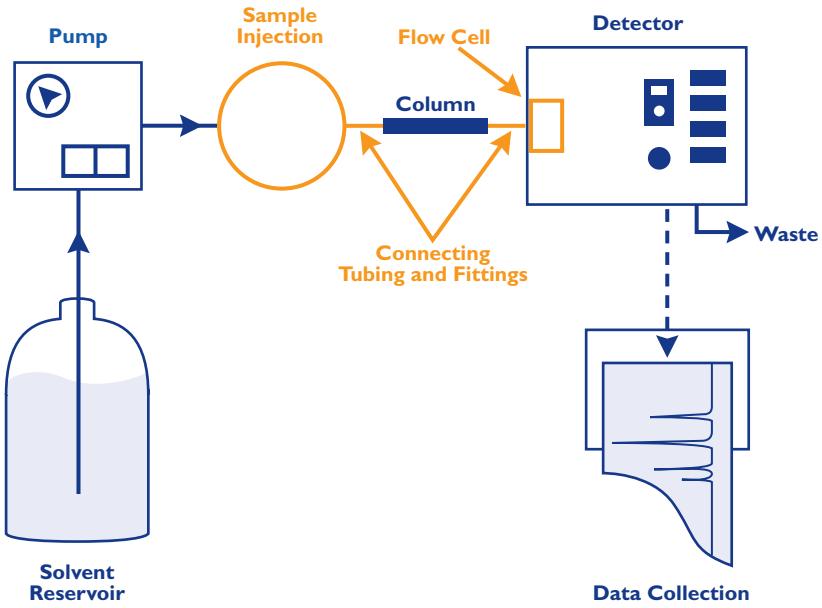
HPLC Equipment Considerations

In Figure B we suggest how to estimate column back pressure for an ultra-fast column and determine if it is within the maximum acceptable operating pressure of your LC equipment. Other important equipment considerations when using an ultra-fast column are the HPLC system's extra column volume (ECV), detector response time, and data collection rate. Peaks elute from ultra-fast columns with much smaller peak volumes than with conventional columns. To gain the maximum advantage from ultra-fast columns when using conventional equipment, modifications may have to be made to minimize ECV. In addition, the detector time constant and data collection rate should be set fast enough to collect sufficient peak information (A good rule of thumb is 20 points per peak.) You can still gain significant benefits using a variety of ultra-fast column geometries, without making any changes to conventional equipment, but you could be sacrificing much of the resolving power that these columns offer.

Extra Column Volume

The volume in an HPLC system external to the column that contributes to the total peak volume is referred to as extra column volume, or ECV. It typically comes from the sample injector, including the volume of sample injected, the tubing connecting the sample injector to the column, the tubing connecting the column to the detector, the detector flow cell, plus any volume added by heat exchangers, fittings, connectors and inline filters (Figure G). In general, you want to keep the ECV to less than 1/2 of the total peak volume (at a given k value) to get 90% of the maximum resolution from a column.

Figure G: Extra Column Volume (ECV)



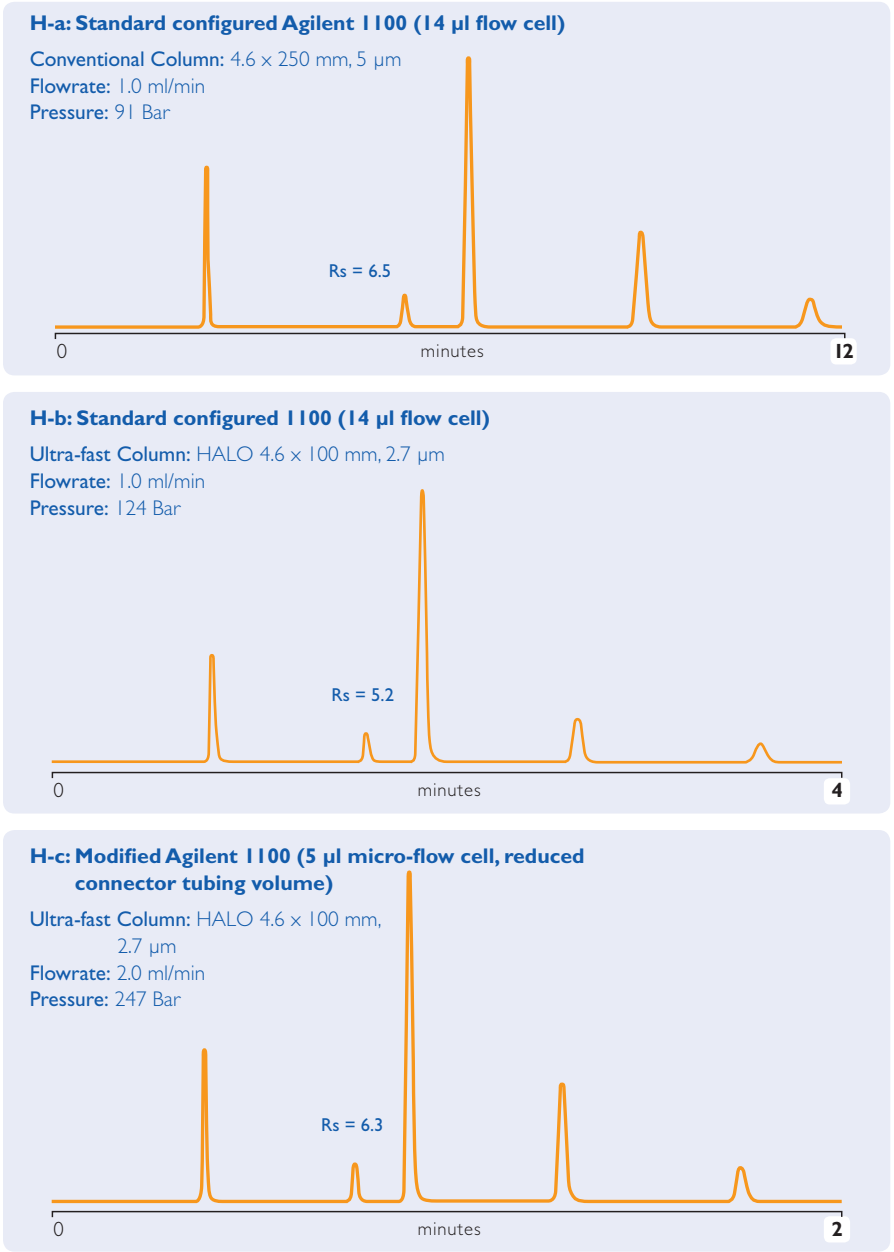
ECV, Extra Column Volume = sample volume + connecting tubing volume + fitting volume + detector flow cell volume

The location of extra column volume (ECV) is indicated in this schematic of LC equipment by the gold color.

For gradient separations, one can usually ignore the pre-column contribution to the ECV, except, of course, that this volume does contribute to the gradient delay volume. In a gradient separation (as in an isocratic one), there will be band focusing at the head of the column if the sample solvent is weaker than the starting gradient mobile phase, and if the column is not overloaded in terms of mass or volume. However, for gradient separations, additional band compression can occur for an analyte (unless it has little or no retention in the starting gradient mobile phase) because the ever-increasing stronger solvent at the back of the band accelerates the trailing portion of the band toward the front of the band.

In Figure H the performance of a 250 mm, 5-µm conventional column is compared to that of an ultra-fast HALO column using a quaternary Agilent 1100 in its standard configuration, (ECV ~ 36 µL) and in a configuration that had been optimized for ultra-fast columns. With the quaternary 1100 in its standard configuration the ultra-fast HALO column provides shorter analysis time (Figure H-b), but the resolution is less than one would predict for the HALO column. After reducing ECV, the ultra-fast column delivers a high speed separation and the resolution expected (Figure H-c).

Figure H: Better performance is obtained from ultra-fast columns when conventional LC equipment is modified to reduce ECV.



This ultra-fast column reduces analysis time by 85% while achieving similar resolution compared to the longer conventional column when the LC equipment's ECV is reduced.

Table 4 provides general guidelines for the maximum amount of system ECV for different dimensions of ultra-fast columns. These estimates assume isocratic conditions. For gradient conditions, more ECV may be tolerated if it is located before the column.

Table 4: Recommended equipment ECV, data rate and time constant for maintaining optimum resolving power of ultra-fast columns

Column I.D.(mm)	Column Length (mm)	Total Maximum ECV (µl)	Maximum Volume Contributed by Detector Flow Cell	Minimum 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	10	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	10	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	10	<0.1

By keeping the LC equipment's ECV to less than the volumes listed above, close to optimum performance is achievable for the specific ultra-fast column dimensions indicated. These estimates are based on ultra-fast columns with average plate counts of 220,000 N/Meter, a peak retention factor (k) of 2 and maintaining over 90% of the resolving power of the ultra-fast columns. Even though gradient elution conditions may permit larger total ECV than what is recommended here, the maximum recommended volume for the detector flow cell should still be adhered to.

Table 5: Estimating Capillary Tubing Volume

Tubing Volumes		
ID (inches)	ID (mm)	µL/cm
0.005	0.127	0.13
0.007	0.178	0.25
0.009	0.229	0.41
0.010	0.254	0.51

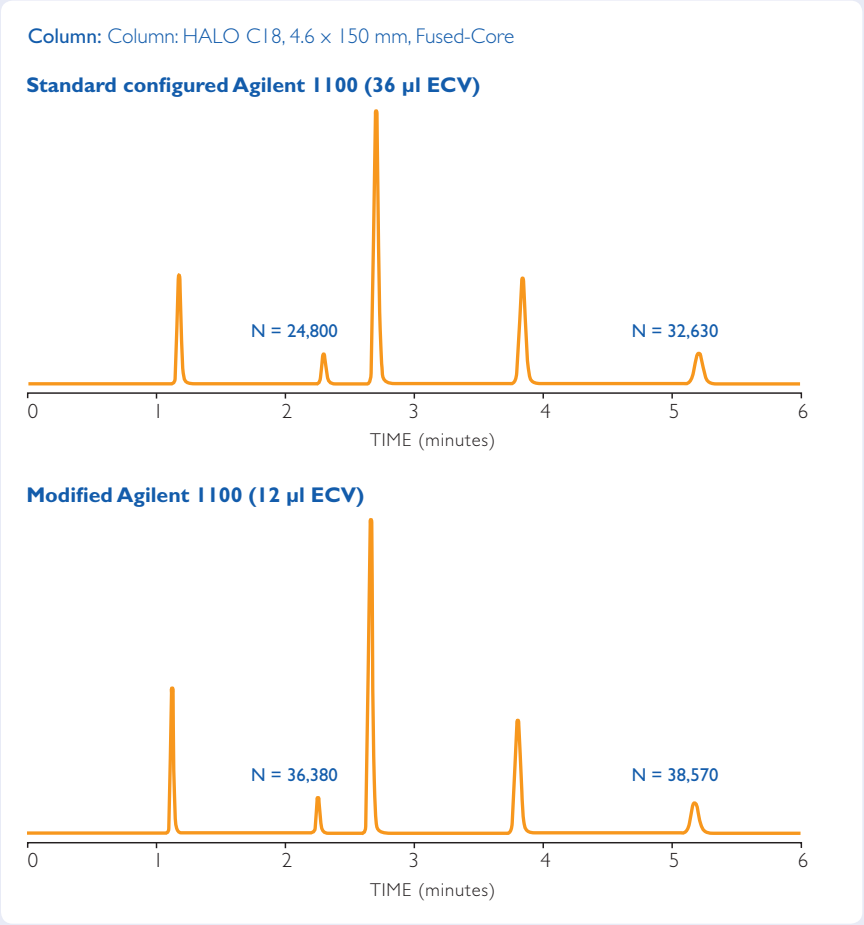
A considerable amount of connector tubing ECV can be reduced by using smaller ID tubing. However, you have to balance this benefit against the increased possibility of buffer precipitation and greater flow restriction from smaller ID tubing. The use of tubing with 0.178 mm (0.007") ID seems to be a good compromise for most situations with 4.6 mm ID columns.

How can you know if your HPLC has too much extra column volume?

It may be possible to obtain ECV data from the equipment manufacturer, but this information is not always reliable. There have been several different approaches suggested for measuring the extra column volume contributed by a piece of equipment, but they have limitations in terms of accuracy and most of them require quite a bit of time investment. We suggest that the time spent in determining an accurate measure of an LC system's extra column volume may not yield results that are significantly more useful than a simple "guesstimate." Such an estimate can be obtained by adding up the obvious known volumes in the system. For example, a standard configured Agilent 1100 would have approximately 36 µl. The standard flow cell contributes 14 µL (13 µL DAD/MWD), an additional 19 µL comes from the connector tubing (0.178 mm ID, 750 mm in length) and 3 µL comes from the heat exchanger.

One way to assess whether you have excessive ECV in your system for a given column is to compare the plate count for an early eluting peak (for example, $k < 2$) to the plate count for a later eluting peak (for example, $k > 4$). If the early eluting peak has a significantly lower plate count (< 80%) than the later eluting peak, then you may want to invest the time to reduce the ECV in your equipment. An example of such an assessment is described in Figure I.

Figure 1: Indication of Excessive ECV



An indication that you may have excessive ECV in your LC equipment for a given column geometry is when the plate count for an early eluting peak is significantly lower than for a late eluting peak. Early eluting peaks have smaller volumes than late eluting peaks and are therefore more affected by ECV. In the standard configured Agilent 1100 (upper trace) the first peak has 24,800 plates, but the last peak has over 32,000 plates. While this is good performance from the column, this finding does hint that much better performance could be achieved with this column by reducing the ECV. Indeed, the second chromatogram shows 36,380 plates for the first eluting peak and 38,570 plates for the last eluting peak using the same column, but after the Agilent 1100 had been modified to reduce the ECV.

Modify conventional HPLC equipment for optimum performance from ultra-fast columns

Sometimes you can improve performance so that you can get acceptable, if not optimum, results by making a few simple changes to instrument parameters. For example, the easiest change to make is to decrease your detector response time (time constant) and/or increase your data collection rate. The next easiest change to make is to reduce your sample injection volume to the minimum amount necessary to achieve acceptable peak response. A third change to make is to determine whether you can make the sample solvent weaker and yet maintain analyte solubility. If these tactics fail to achieve acceptable performance levels, replace the capillary tubing connecting the column to the sample injector and to the detector with smaller ID tubing, and use the minimum length possible. Table 5 contains reference data that will help you to estimate tubing volume. Finally, if you still are not satisfied with the system's performance, replace your detector flow cell with one that has less volume. Table 4 will act as a guide in choosing the appropriate detector flow cell.

Figure J: Recommended consecutive steps to take in modifying conventional LC equipment for use with ultra-fast columns

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.

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.**
   If this is not possible, it will place more restrictions on the volume of sample that you can inject. If you are running gradient conditions, you will likely be able to inject considerably more sample volume than you would under isocratic conditions. However, it is still important to keep the sample solvent weaker than the initial strength of 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.**
   Table 4 provides guidelines for selecting detector flow cell volumes.

Sometimes making significant equipment modifications to achieve optimum performance from an ultra-fast column is not worth the time, trouble and expense when acceptable results can be achieved easier and faster. Here we recommend consecutive steps to take in modifying conventional LC equipment for use with an ultra-fast column, beginning with the easiest and cheapest modifications to make and progressing to more difficult and/or more expensive modifications. Once you have achieved acceptable results, you may wish to forego any further equipment modifications.

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