Technical note | 70828

Thermo Fisher SCIENTIFIC

Liquid chromatography

A universal tool for method transfer from HPLC to UHPLC

Authors
Holger Franz and Susanne Fabel; Thermo Fisher Scientific, Germering, Germany

Keywords
Method speed-up, method acceleration, solvent saving, time saving, small particle columns

Introduction

With the commercialization of ultra high performance liquid chromatography (UHPLC), there has been a continuing trend towards this technology's use. This trend is mainly driven by innovations in liquid chromatography instrumentation and column packing. Compared to high performance liquid chromatography (HPLC), column particle sizes are smaller, down to the sub-2 µm range, and provide more theoretical plates and resolution than columns of the same length that use larger-sized particles.

However, when transferring methods from HPLC to UHPLC, it is usually sufficient to maintain the resolution of the original method. Therefore, a popular strategy is to use smaller particles in shorter columns—this approach maintains resolution and provides faster separations. Rather complex calculations are required to adapt parameters, such as flow rate, injection volume, or gradient profile to the new column characteristics. Thermo Fisher Scientific offers online and software-embedded tools for easy method transfer from HPLC to UHPLC. Optimal instrument settings are automatically calculated based on known parameters of the conventional HPLC application.

This work presents the theoretical background and introduces the equations for an application transfer to UHPLC and describes the online Thermo Scientific method transfer tool. With a focus on the online method transfer tool, this technical note explains how to enter application details, and will familiarize you with the calculated results. The tool provides valuable features beyond the basic calculations to deal with changing gradient delay volume (GDV), the adaptation of data collection rates, and recommended reconditioning times.

Method acceleration strategy

The purpose of accelerating a typical method is to achieve sufficient resolution in the shortest possible time. The strategy is to maintain the resolving power of the method by using shorter columns packed with smaller particles. The theory for this approach is based on chromatographic mechanisms, found in almost every chromatography text book. The following fundamental chromatographic equations are applied by the method transfer tools for translating methods from HPLC to UHPLC with fully-porous particle columns of similar chromatographic selectivity.

The separation efficiency of a method is stated by the peak capacity *P*, which describes the number of peaks that can be resolved in a given time period. A common definition of the peak capacity is the run time divided by the average peak width. Hence, a small peak width is essential for a fast method with high separation efficiency. The peak width is proportional to the inverse square root of the number of theoretical plates *N* generated by the column. Taking into account the length of the column, its efficiency can also be expressed by the height equivalent to a theoretical plate *H*. The relationship between plate height *H* and plate number *N* of a column with the length *L* is given by (Equation 1).

Equation 1: $N = \frac{L}{H}$

Where:
N = Plate number
L = Column length
H = Plate height

Low Height equivalents will therefore generate a high number of theoretical plates, and hence small peak width for high peak capacity is gained. But which factors define *H*? For an answer, the processes inside the column have to be considered, which are expressed by the Van Deemter equation (Equation 2).

Equation 2: $H = A + \frac{B}{u} + C \cdot u$

Where:
u = Linear velocity
A = Eddy diffusion
B = Longitudinal diffusion
C = Resistance to mass transfer

The eddy diffusion A describes the mobile phase movement along different random paths through the stationary phase, resulting in broadening of the analyte band. The longitudinal diffusion of the analyte against the flow rate is expressed by the term *B*. Term *C* describes the resistance of the analyte to mass transfer into the pores of the stationary phase. This results in higher band broadening with increasing velocity of the mobile phase. The well known Van Deemter plots of plate height *H* against the linear velocity of the mobile phase are useful in determining the optimum mobile phase flow rate for highest column efficiency with lowest plate heights. A simplification of the Van Deemter equation, according to Halász[1] (Equation 3) allows a simple estimation of column efficiency for fully porous particles.

Equation 3: $H = 2 \cdot d_p + \frac{6}{u} + \frac{d_p^2 \cdot u}{20}$

Where:
d_p = Particle size (in µm)
u = Velocity of mobile phase (in mm/s)

The plots of plate height *H* against velocity u depending on the particle sizes d_p of the stationary phase (see Figure 1, top) visually demonstrate the key function of small particle sizes in the method acceleration strategy: the smaller the particles, the smaller the plate height and therefore the better the separation efficiency. An efficiency equivalent to larger particle columns can be achieved by using shorter columns and therefore shorter run times.

Another benefit with using smaller particles is shown for the 2 µm particles in Figure 1: Due to improved mass transfer with small particle packings, further acceleration of mobile phases beyond the optimal flow rate with minimal change in the plate height is possible.

Optimum flow rates and minimum achievable plate heights can be calculated by setting the first derivative of the Halász equation to zero. The optimal linear velocity (in mm/s) is then calculated by Equation 4.

Equation 4: $u_{opt} = \sqrt{\frac{B}{C}} \approx \frac{10}{d_p}$

Where:
u_{opt} = Optimum linear velocity (in mm/s)

The minimum achievable plate height as a function of particle size is calculated by insertion of Equation 4 in Equation 3, resulting in Equation 5.

Equation 5: $H_{min} \approx 3 \cdot d_p$

Where:
H_{min} = Plate height at minimum

Chromatographers typically prefer resolution over theoretical plates as a measure of the separation quality. The achievable resolution R of a method is directly proportional to the square root of the theoretical plate number as can be seen in Equation 6.

Equation 6: $R = \frac{1}{4} = \cdot \sqrt{N} \cdot \frac{k_2}{1 + k_2} \cdot \frac{\alpha - 1}{\alpha}$

Where:
R = Resolution
k_2 = Retention factor
α = Selectivity

If the column length is kept constant and the particle size is decreased, the resolution of the analytes improves. Figure 1, bottom, demonstrates this effect using 5 µm and 2 µm particles.

When transferring a gradient method, the scaling of the gradient profile to the new column format and flow rate has to be considered to maintain the separation performance. The theoretical background was introduced by Snyder[2] and is known as the gradient volume principle. The gradient volume is defined as the mobile phase volume that flows through the column at a defined gradient time t_G. Analytes are considered to elute at constant eluent composition provided the gradient volume is not changed relative to the column volume. Keeping the ratio between the gradient volume and the column volume constant therefore results in a correct gradient transfer to a different column format.

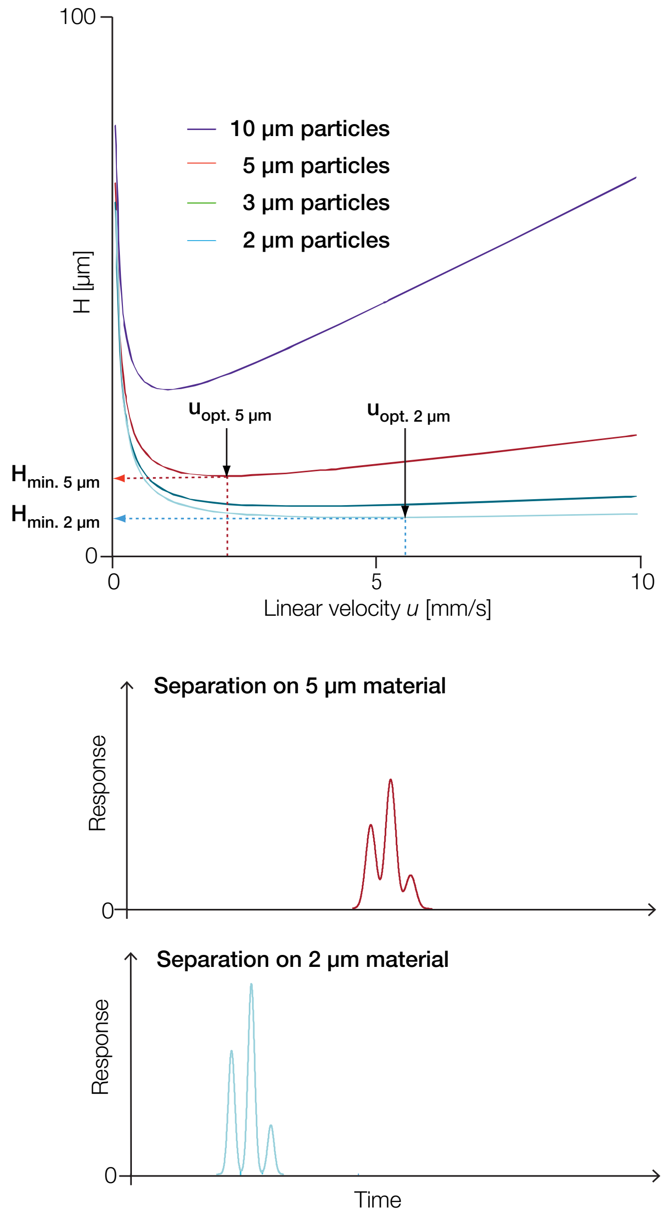

Figure 1. Smaller particles provide more theoretical plates and more resolution, demonstrated by the improved separation of three peaks (bottom) and smaller minimum plate heights *H* in the Van Deemter plot (top). At linear velocities higher than $u_{opt,}$ *H* increases more slowly when using smaller particles, allowing higher flow rates and therefore faster separations while keeping separation efficiency almost constant. The acceleration potential of small particles is revealed by the Van Deemter plots (top) of plate height *H* against linear velocity *u* of mobile phase: Reducing the particle size allows higher flow rates and shorter columns because of the decreased minimum plate height and increased optimum velocity. Consequently, smaller peak width and improved resolution are the results (bottom).

Taking into account the changed flow rates F and column volume, the gradient time intervals t_G of the new methods are calculated with Equation 7.

$$\text{Equation 7: } t_{G,\,new} = t_{G,\,old} \cdot \frac{F_{old}}{F_{new}} \cdot \frac{L_{new}}{L_{old}} \cdot \left(\frac{d_{c,\,new}}{d_{c,\,old}}\right)^2$$

Where:

t_G = Gradient time
F = Flow rate
d_c = Column diameter

An easy transfer of method parameters can be achieved by using the online HPLC Method Transfer Calculator (Figure 2), which automatically applies the discussed theory.

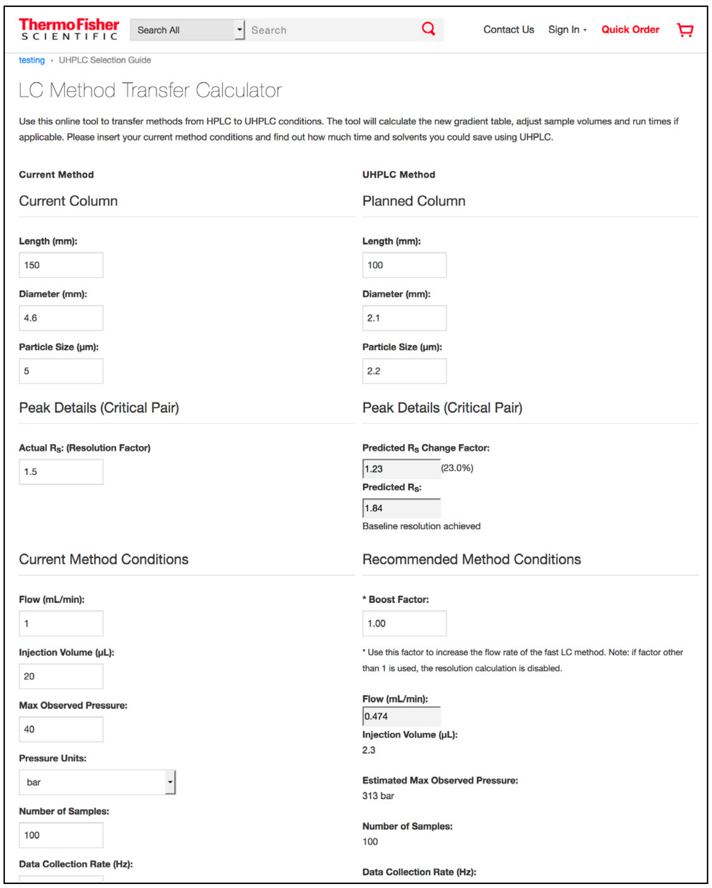

Figure 2. The online Thermo Scientific HPLC Method Transfer Calculator transfers a conventional (current) HPLC method to a new (planned) UHPLC method.

Prerequisites

The method transfer tool is an universal tool and can be used with any HPLC system. Nevertheless, some prerequisites have to be considered for a successful method transfer, which is demonstrated in this technical note by the separation of seven soft drink additives.

Column dimension

First, the transfer of an HPLC to a UHPLC method requires the selection of an adequate column filled with smaller particles. The UHPLC method is predicted best if the selectivity of the stationary phase is maintained. Therefore, a column from the same manufacturer and with nominally identical surface modification is favored for an exact method transfer. If this is not possible, a column with the same nominal stationary phase is the next best choice. The separation is made faster by using shorter columns, but the column should still offer sufficient column efficiency to allow at least a baseline separation of analytes. Table 1 gives an overview of the theoretical plates expected by different column length and particle diameter size combinations using Thermo Scientific™ Acclaim™ 120 C18 column particle sizes.

Table 1. Theoretical plates depending on column length and particle diameter (calculated using Equation 5).

	Theoretical plates N		
Column length (mm)	Particle size		
	5 µm	3 µm	2.2 µm
250	17,100	28,000	38,000
150	9,900	17,000	23,000
100	6,660	11,000	15,000
75	5,040	8,300	11,000
50	3,330	5,600	7,600

If the resolution of the original separation is higher than required, columns can be shortened. Keeping the column length constant while using smaller particles improves the resolution. Reducing the column diameter does not shorten the analysis time but decreases mobile phase consumption and sample volume. Taking into account an elevated temperature, smaller column inner diameters reduce the risk of thermal mismatch.

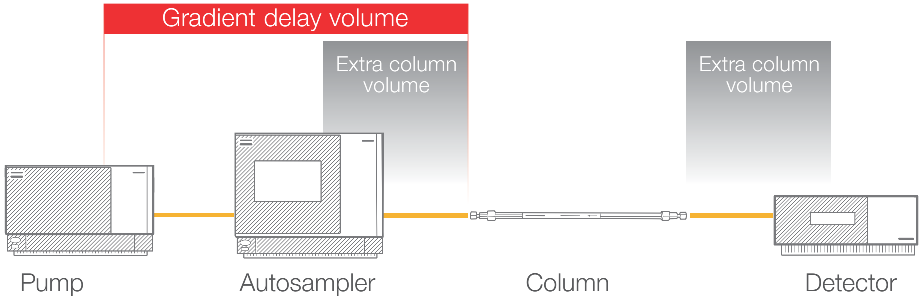

Figure 3. Gradient delay volume and extra column volume of an HPLC system. Both play an important role in method acceleration.

System requirements

Smaller particles generate higher backpressure. The linear velocity of the mobile phase has to be increased while decreasing the particle size to work within the Van Deemter optimum. The Thermo Scientific™ LC platforms support this approach. The Thermo Scientific™ Vanquish™ UHPLC platform provides the system pressure, flow range and low dispersion capillary connections to allow the method speed-up. These pressure capabilities provide the potential to accelerate applications even further by increasing the flow rate.

For fast gradient methods, the gradient delay volume (GDV) plays a crucial role. The HPLC Method Transfer Calculator follows the gradient volume principle introduced by L. Snyder.[2] The gradient volume is defined as the mobile phase volume that flows through the column in a defined gradient time or t_G. Analytes are considered to elute at a constant eluent composition. Therefore, keeping the ratio constant between the gradient volume and the column volume results in a correct gradient transfer to a different column format. To achieve this, the gradient delay volume (GDV) of the system must also follow the gradient volume principle (Equation 8).

Equation 8: $$V_{GDV,\ new} = \frac{V_{GDV,\ old} \cdot V_{column,\ new}}{V_{column,\ old}}$$

Where:

V_{GDV} = Gradient delay volume

V_{column} = Column volume

The GDV is defined as the volume from the first point of mixing to the head of the column (Figure 3). The main contributors to the GDV are the pump-mixing volume, the autosampler fluidics, and all connection capillaries that are in front of the column. The authors recommend the determination of the GDV with the method described in Reference 3.

Scaling the GDV down by the same factor as the column volume fulfills the requirements of the gradient volume principle and maintains the selectivity of the original method[4] (it is assumed that the total porosity is constant for both columns).

In practice, it is difficult to precisely scale the GDV of the system. It is necessary to scale down the mixing volume of the pump in direct proportion to the column volume, as this is the biggest contributor to the total GDV. To address this, Vanquish UHPLC pumps have been designed to provide the flexibility required, offering a highly customizable two-step mixing-volume concept (Figure 4). In addition to the pump mixing volume adaption, the Vanquish Autosampler allows the fine-tuning of the GDV by adjusting the metering device.

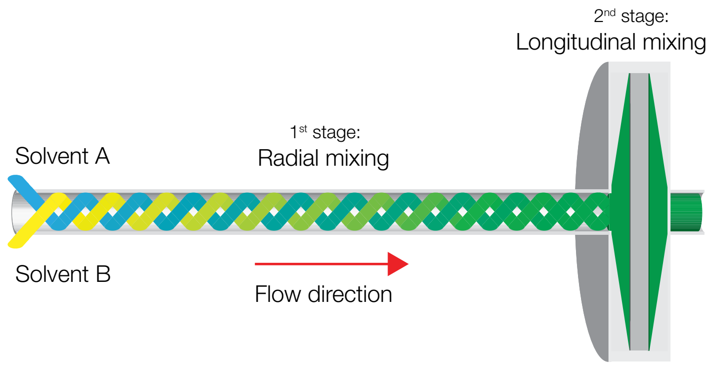

Figure 4. The highly customizable two-step mixing concept of the Vanquish UHPLC series allows adapting the GDV to individual needs.

Besides the gradient delay volume, the extra column volume is an important parameter for fast LC methods. The extra column volume is the volume in the system through which the sample passes and hence contributes to the band broadening of the analyte peak (Figure 3). The extra column volume of an optimized LC system should be below $^1/_{10}$th of the column volume. Therefore the length and inner diameter of the tubing connections from injector to column and column to detector should be as small as possible. To avoid dead volumes, special care has to be taken while installing the fittings. Thermo Scientific™ Viper™ connectors provide near zero-dead volume by sealing at the tubing tip, hence ensuring optimized connections of conventional HPLC and modern UHPLC systems without any additional tools. Even though Viper withstands UHPLC backpressures of up to 1500 bar (22,000 psi), it is a fingertight fitting system which requires only small torques to seal. In addition to the correct tubing connections, the volume of the flow cell has to be adapted to the peak volumes eluting from the UHPLC column. In general, extra-column band broadening will be insignificant if the flow cell volume is no larger than approximately 10% of the smallest peak volume.[5,6]

Detector settings

When transferring a conventional method to a UHPLC method, the detector settings have a significant impact on the detector performance. The data collection rate and time constant have to be adapted to the narrower peak shapes. In general, each peak should be defined by at least 30 data points. The data collection rate and time constant settings are typically interrelated to optimize the amount of data points per peak and reduce short-term noise while still maintaining peak height, symmetry, and resolution. The Thermo Scientific HPLC Method Transfer Calculator has a function to estimate the data collection rate of the new method based on the old data collection rate and used column dimension and particle size. Details on this function are explained in the Instrument Settings Section of this technical note.

Alternatively to the estimation of the HPLC Method Transfer Calculator, the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software has a wizard to automatically calculate the best settings, based on the input of the minimum peak width at half height of the chromatogram. By using the embedded method speed-up tool in Chromeleon CDS, the correct data collection rate is automatically calculated during the walk through of method speed-up steps by relying on the data recorded with the original method. Please refer to the detector operation manual for further details.

Method acceleration using the transfer tool

Separation example

In this separation example, a standard mixture of 7 common soft drink additives was separated by gradient elution at 45°C on two different columns, and with a UHPLC system with binary pump. Chromeleon CDS was used for both controlling the instrument and reporting the data. A standard mixture of seven common soft drink additives was separated by gradient elution at 45°C on two different columns:

- Conventional HPLC Column: Thermo Scientific™ Acclaim™ 120, C18, 5 µm, 4.6 × 150 mm column, (P/N 059148)
- UHPLC Column: Thermo Scientific™ Acclaim™ RSLC 120, C18, 2.2 µm, 2.1 × 50 mm column (P/N 068981)

With the HPLC column, the data collection rate was 5 Hz, with the UHPLC column, data collection rates were 25 Hz and 50 Hz. UV absorption was measured at 210 nm. Further method details such as flow rate, injection volume, and gradient table of conventional and accelerated methods are described in the following section. The parameters for the method transfer were calculated with the HPLC Method Transfer Calculator.

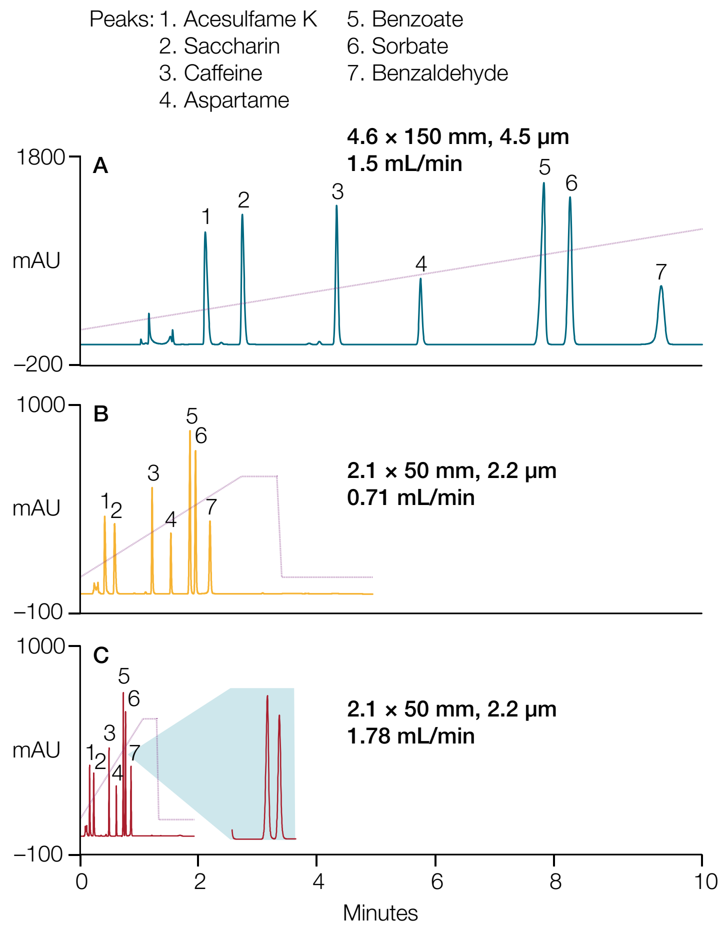

Figure 5. Method acceleration with the HPLC Method Transfer Calculator from A) a conventional LC separation on a Acclaim 120 C18 5 µm particle column, to B) and C) UHPLC separations on a Acclaim RSLC 120 C18 2.2 µm particle column.

The conventional separation of seven soft drink additives is shown in Figure 5A. With the HPLC Method Transfer Calculator, the method was moved successfully to UHPLC methods (Figure 5B and 5C) at two different flow rates. The easy transfer with this universal tool is described in the following paragraph.

Column selection for appropriate resolution

The column for method acceleration must provide sufficient efficiency to resolve the most critical pairs. In this example, separating peaks 5 and 6 is most challenging. An initial selection of the planned column dimensions can be made by considering the theoretical plates according to Table 1. The 4.6 × 150 mm, 5 µm column provides approximately 9,900 theoretical plates. On this column, the resolution is $R(5,6)$=3.48. This resolution is sufficiently high to select a fast LC column with fewer theoretical plates. Therefore, a 2.1 × 50 mm, 2.2 µm column with approximately 7600 plates was selected.

The first values to be entered into the HPLC Method Transfer Calculator are the current column dimension, planned column dimension, and the resolution of the critical pair. Based on the assumption of unchanged stationary phase chemistry, the calculator then predicts the resolution provided by the new method (Figure 6).

In the example in Figure 6, the predicted resolution between benzoate and sorbate is 2.89. With a resolution of R ≥ 1.5, the message "Baseline resolution achieved" pops up. This indicates that a successful method transfer with enough resolution is possible with the planned column. If R is smaller than 1.5, the red warning "Baseline is not resolved" appears. Note that the resolution calculation is performed only if the boost factor (*BF*) is 1, otherwise it is disabled. The function of the *BF* is described in the Adjust Flow Rate section.

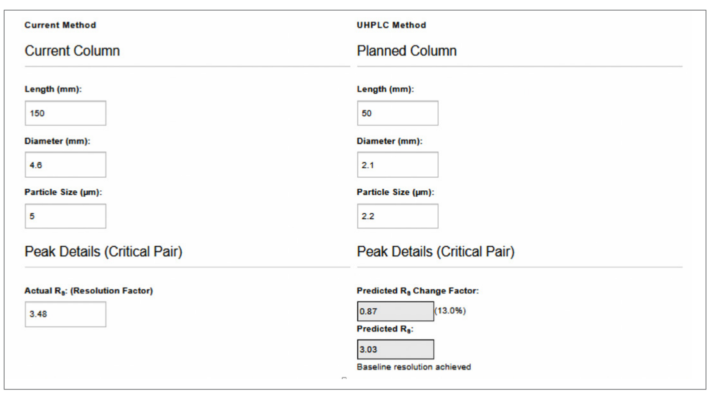

Figure 6. Column selection considering the resolution of the critical pair.

Instrument settings

The next section of the HPLC Method Transfer Calculator considers basic instrument settings. These are flow rate, injection volume, system backpressure, and data collection rate of the current method (Figure 7). Furthermore, the throughput gain with the new method can be calculated if the number of samples to be run is entered.

Adjust flow rate

As explained by Van Deemter theory, smaller particle phases need higher linear velocities to provide optimal separation efficiency. Consequently, the HPLC Method Transfer Calculator automatically optimizes the linear velocity by the ratio of particle sizes of the current and planned method. In addition, the new flow rate is scaled to the change of column cross section if the column inner diameter changed. This keeps the linear velocity of the mobile phase constant. A *BF* can be entered to multiply the flow rate for a further decrease in separation time. If the calculated resolution with *BF*=1 predicts sufficient separation, the method can be accelerated by increasing the *BF* and therefore increasing the flow rate. Figure 1 shows that applying linear velocities beyond the optimum is no problem with smaller particle phases, as they do not significantly lose plates in this region.

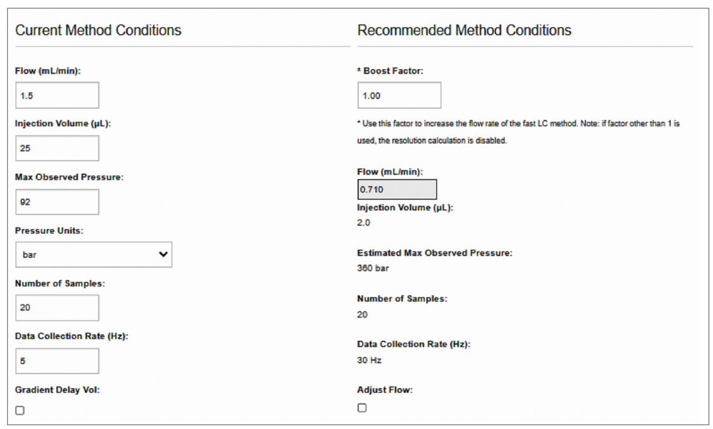

Figure 7. The flow rate, injection volume, backpressure, and data collection rate of the current method are scaled to the new column dimension.

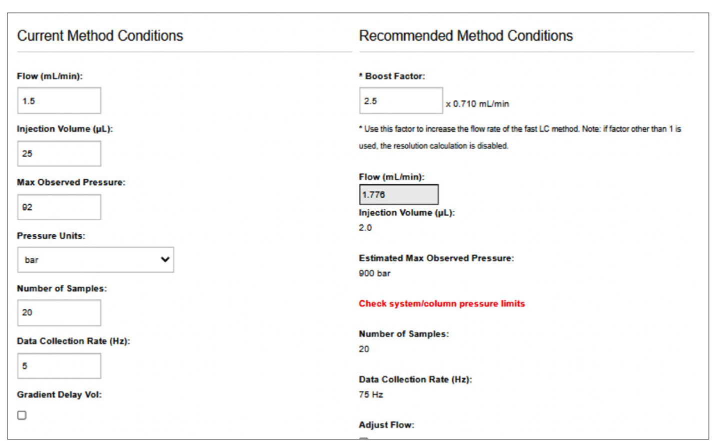

Figure 8. The new flow rate is further accelerated by applying the Boost Factor of 2.5.

For the separation at hand, the flow rate is scaled from 1.5 mL/min to 0.639 mL/min when changing from an Acclaim 120 C18 4.6 × 150 mm, 5 µm column to a 2.1 × 50 mm, 2.2 µm column (Figure 7), adapting the linear velocity to the column dimensions and the particle size. The predicted resolution between peak 5 and 6 for the planned column is *R*=2.89. The actual resolution achieved is *R*=2.91, almost as calculated (chromatogram B in Figure 5).

A *BF* of 2.5 was entered for further acceleration of the method (Figure 8). The method was then performed with a flow rate of 1.599 mL/min, and resolution of the critical pair was still sufficient at R=2.56 (see zoom in chromatogram C in Figure 5).

Note that the HPLC Method Transfer Calculator shows the warning "Check system/column pressure limits" at estimated pressure beyond 8700 psi (600 bar). As the tool can be used with any LC instrument and column, it is our goal to spare you from accidentally applying pressures that are too high. Although UHPLC is an established technology today, many so-called UHPLC columns remain incompatible with pressures beyond 8,700 psi.

Data collection rate

A typical peak requires 30 data points for accurate and precise integration. A method transfer from HPLC to UHPLC columns typically reduces both the peak volume and the peak width. To meet the 30 data points requirement, the data collection rate must be adjusted.

The HPLC Method Transfer Calculator calculates the data collection rate of the new method based on the current data rate and both column dimensions entered (Equation 9). It is assumed that the current data rate setting is suitable for the given separation. In the example at hand, the data collection rate changes from 5 Hz to 64 Hz (Figure 9).

Equation 9: $$D_{new} = \frac{D_{old}}{\sqrt{\dfrac{L_{new} \cdot d^3_{p,\,new}}{L_{old} \cdot d^3_{p,\,old}}}}$$

Where:

V_{GDV} = Adjusted data collection rate (Hz)

D = Data collection rate (Hz)

L = Column length (mm)

d_p = Particle size (µm)

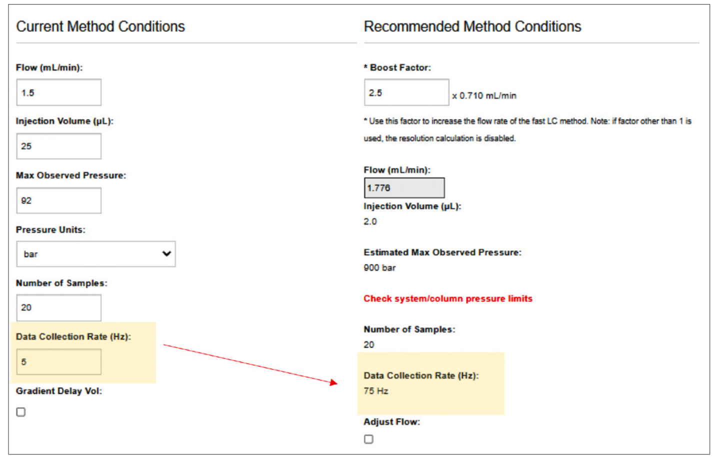

Figure 9. An example of the adjusted data acquisition rate using the Thermo Scientific Method Translate Tool.

Scale injection volume

The injection volume has to be adapted to the new column dimension to achieve similar peak heights by equivalent mass loading. Therefore the injection plug has to be scaled to the change of column cross section. In addition, shorter columns with smaller particles cause a reduced zone dilution. Consequently, sharper peaks compared to longer columns are expected. The new injection volume is then calculated by Equation 10, taking a changed cross section and reduced band broadening by modified particle diameter into account.

Equation 10: $$V_{inj,\,new} = V_{inj,\,old} \cdot \left(\frac{d_{c,\,new}}{d_{c,\,old}}\right)^2 \cdot \sqrt{\frac{L_{new} \cdot d_{p,\,new}}{L_{old} \cdot d_{p,\,old}}}$$

Where:

V_{inj} = Injection volume

d_p = Particle size (µm)

d_c = Column diameter (mm)

Generally, it is recommended that a smaller flow cell is used with the UHPLC method to minimize the extra column volume. Depending on the manufacturer and the type of detector, such a flow cell may come with a shorter light path, directly influencing the response of the detector. This potential difference is not considered by the method transfer tool. In the example of the soft drink analysis, the injection volume is scaled from 25 µL to 2.1 µL when replacing the Acclaim 120 C18 4.6 × 150 mm, 5 µm column with a 2.1 × 50 mm, 2.2 µm column (Figure 7).

Predicted backpressure

Accelerating the current method by decreasing particle size and column diameter and increasing flow rate means elevating the maximum generated backpressure. The pressure drop across a column can be approximated by the Kozeny-Carman formula.[7] The pressure drop of the new method is predicted by the HPLC Method Transfer Calculator considering changes in column cross section, flow rate, and particle size and is multiplied by the boost factor. The viscosity of mobile phase is considered constant during method transfer. The calculated pressure is only an approximation and does not take into account nominal and actual particle size distribution depending on column manufacturer.

In the example of the soft drink analysis, the actual pressure increases from 92 bar to 182 bar (1334 psi to 2,640 psi) with *BF*=1 on the 2.1 × 50 mm column, and to 460 bar (6,671 psi) for the UHPLC method with *BF*=2.5. The pressures predicted by the HPLC Method Transfer Calculator are 262 bar and 656 bar (3,800 psi and 9514 psi), respectively. The pressure calculation takes into account the change of the size of the column packing material. In a method transfer situation, the pressure is also influenced by other factors such as particle size distribution, system fluidics pressure, change of flow cell, etc. When multiplication factors such as the boost factor are used, the difference between calculated and real pressure is pronounced. The pressure calculation is meant to give guidance on, what flow rates might be feasible on the planned column. However, it should be confirmed by applying the flow on the column.

Adapt gradient table

The gradient profile has to be adapted to the changed column dimensions and flow rate following the gradient-volume principle. The gradient steps of the current method are entered into the Current Gradient Table section. The calculator then scales the gradient step intervals appropriately and creates the Planned Gradient Table of the new method further down the page.

The adapted gradient table for the soft drink analysis while using a boost factor *BF*=1 is shown in Figure 10. According to the gradient-volume principle, the total run time is reduced from 29.0 min to 4.729 min by taking into account the changed column volume from a 4.6 × 150 mm, 5 µm to a 2.1 × 50 mm, 2.2 µm column and the flow rate reduction from 1.5 mL/min to 0.639 mL/min. The separation time was further reduced to 1.890 min by using boost factor *BF*=2.5. Gradient time steps were adapted accordingly. The comparison of the peak elution order displayed in Figure 5 shows that the separation performance of the gradient was maintained during method transfer.

Current Gradient Table

Step	Time (min)	%A	%B	%C	%D
1	0.00	93.0	7	0.0	0.0
2	16	50.0	50	0.0	0.0
3	19.5	50.0	50	0.0	0.0
4	20	93.0	7	0.0	0.0
5	29	93.0	7	0.0	0.0
6					
7					
8					
9					
10					

End Time: 29.000

Recommended Reconditioning Time: 6.481

Planned Gradient Table

Time (min)	%A	%B	%C	%D
0.00	93.0	7	0	0
0.939	50.0	50	0	0
1.144	50.0	50	0	0
1.173	93.0	7	0	0
1.702	93.0	7	0	0

End Time: 1.702

Recommended Reconditioning Time: 0.380

Figure 10. The gradient table of the current method (A) is adapted to the boosted method (B) according to the gradient-volume principle.

Additional features of the HPLC Method Transfer Calculator

In addition to the fluidical adaption of the GDV as described in the System Requirement section of this technical note, the HPLC Method Transfer Calculator can also compensate for GDV differences. To do that, activate the check box with the “Gradient Delay Volume” in the tool. A new line shows up now in which the GDV can be entered for both the current and planned method. The assumption here is that the current application runs on a quaternary system with a GDV of 1000 µL. The *BF*=1 application will be transferred to a binary system with 400 µL GDV. The calculator compares this value against the optimal GDV (Equation 11).

$$\text{Equation 11: } t_D = \frac{V_{GDV} - V_{GDV,\,opt}}{F}$$

Where:

t_D = Time shift for injection delay and/or additional gradient steps (min)

$V_{GDV,\,opt}$ = Optimum gradient delay volume (µL)

V_{GDV} = Entered gradient delay volume (µL)

F = Flow rate (µL/min)

A large GDV, as in this example, has an impact on both the moment the gradient takes effect on the column and the equilibration time. Consequently, the calculator suggests delaying the injection by applying a gradient prestart of 0.517 min and extending the equilibration time. Note that the recommended time shift of the injection and the length of the final equilibration step are the same in the example in Figure 11: 0.517 min injection delay equals gradient step 4.729 to 5.246 min.

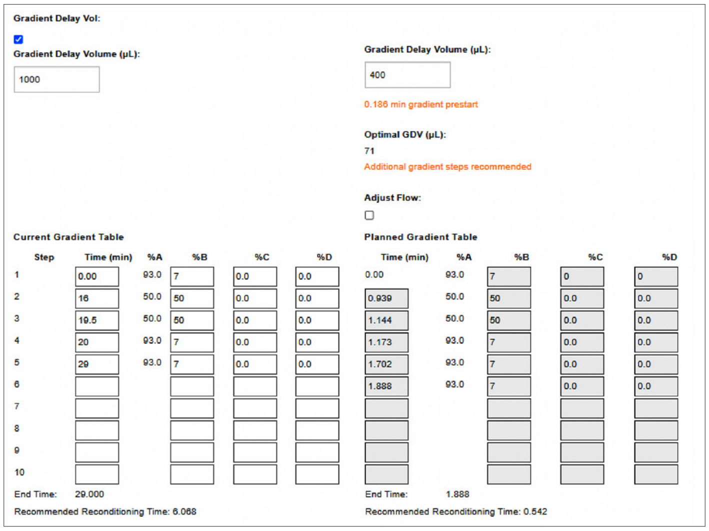

Figure 11. Delayed injection and increased equilibration time for the planned method with a larger GDV.

The tool indicates the optimum GDV to be 70 µL for the new application. With a GDV of 50 µL, (i.e., smaller than the optimum GDV), gradients take effect earlier on the column compared to the original method. By using Equation 11, the HPLC Method Transfer Calculator can automatically compensate for low GDVs by delaying all gradient step times. If the linear gradient starts at 0 min, the calculator then introduces an isocratic hold step after the injection (Figure 12). The HPLC Method Transfer Calculator therefore ensures that users can identify the target GDV and compensate small differences for a seamless method transfer. It is important to note that according to the gradient volume principle, the extra column volume must be scaled down by the same factor as the GDV. The extra column volume is defined as the volume between the sampler and the detector but without the column. In practice, the diameter of all connection tubings after the autosampler must be reduced to a minimum. This assures good support of UHPLC columns even with conventional HPLC instruments.

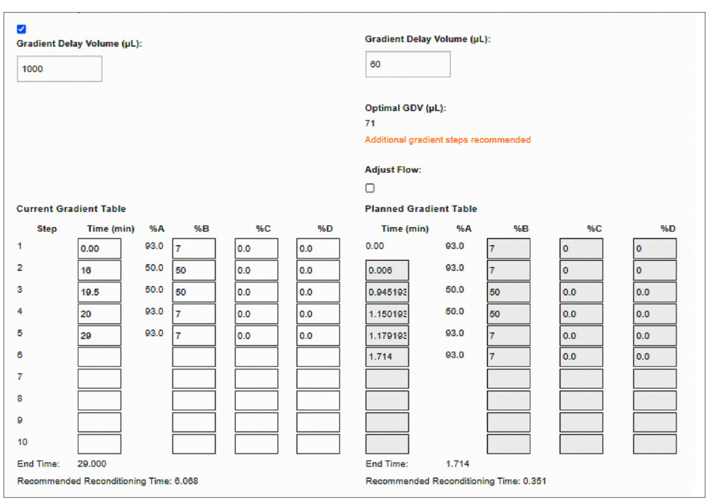

Figure 12. The HPLC Method Transfer Calculator automatically compensates for low GDVs by extending the isocratic hold after the injection. A gradient step is added if the linear gradient starts at 0 min.

Recommended reconditioning time

The calculator suggests a reconditioning time based on the entered column conditions (Figure 13). The suggested reconditioning times are optimized for typical reversed-phase gradient applications. Challenging gradient applications may require significantly longer equilibration.

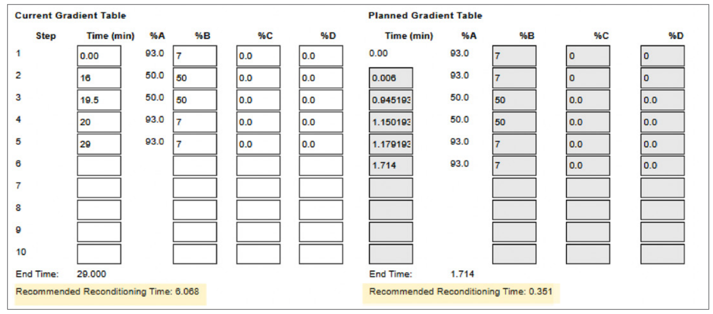

Figure 13. The recommended reconditioning time appears below the gradient table.

The calculation varies between two different scenarios. Without gradient delay volume consideration use Equation 12:

Equation 12: $$t_{Reg} = \frac{5 \cdot CV \cdot \varepsilon_T}{F}$$

Where:

t_{Reg} = Reconditioning time (min)
CV = Geometrical column volume (mL)
ε_T = 0.65; average total porosity

With gradient delay volume consideration for the reconditioning time use Equation 13:

Equation 13: $$t_{Reg} = \frac{5 \cdot CV \cdot \varepsilon_T + GDV}{F}$$

Where:

t_{Reg} = Reconditioning time (min)
CV = Geometrical column volume (mL)
ε_T = 0.65; average total porosity

Consumption and savings

Accelerating your methods has several advantages: to separate analyte peaks faster, and at the same time reduce the mobile phase, and sample volume consumption. Those three advantages are indicated in the method transfer tools. In the method transfer tool the advantages are listed right below the gradient table. The absolute values for the time, eluent, and sample usage are calculated taking the numbers of samples entered into the current instrument settings section of the calculation sheet into account (Figure 14).

Figure 14. The absolute values for analysis time, eluent usage, and sample usage of the current and planned method are calculated by the HPLC Method Transfer Calculator. The savings of eluent, sample, and time due to the method transfer are highlighted.

Regarding the soft drink analysis example, geometrical scaling of the method from the conventional column to the UHPLC method means saving 92% of eluent and 91% of sample. The sample throughput increases 6.1-fold using *BF*=1. The higher flow rate at *BF*=2.5 results in a 15.1-fold increased throughput compared to the conventional LC method (Figure 14).

Conclusion

This technical note teaches the theoretical background required for method transfer, mainly from HPLC to UHPLC. The rather complex relationships between the different equations are easily accessible through the Thermo Scientific HPLC Method Transfer Calculator. Beyond the basic parameters, it provides valuable features on how to deal with changing gradient delay volume, the adaption of data collection rates and recommended column reconditioning times, making it to a valuable tool for any HPLC or UHPLC user. This free tool is mobile friendly and can be found at HPLC Method Transfer Calculator.

References

1. Halász, I.; Endele, R.; Asshauer, J. *J. Chromatogr.,* A **1975**, 112, 37–60.
2. Snyder, L.R.; Dolan, J.W.; Grant, J.R. *J. Chromatogr.,* A **1979**, 165, 3–30.
3. Gilroy, J.J.; Dolan, J.W. *LC/GC Europe,* **2004**, 17(11), 566–572.
4. Schellinger, A.P.; Carr, P.W. *J. Chromatogr.,* A 2005, 1077, 110–119.
5. Poole, C.F. *The Essence of Chromatography;* Elsevier Science: Amsterdam, The Netherlands, 2003; pp 44–46.
6. Hanai, T.T. HPLC: *A Practical Guide;* The Royal Society of Chemistry: Cambridge, U.K., **1999**; pp 25–26.
7. Bear, J. *Dynamics of Fluids in Porous Media;* Dover: Mineola, NY, **1988**.

Learn more at **thermofisher.com/vanquish**