

## Method transfer calculator – Introduction, instructions and help

### Notes on data entry

#### Isocratic methods

Necessary parameters to be entered by the user are the initial column geometry (length, internal diameter and particle size) and method parameters (flow rate, injection volume and analysis time). The pressure may also be entered, if known. The new column dimensions and particle size also must be entered.

The new method parameters (flow rate, injection volume and analysis time) are automatically calculated and displayed. A relative comparison of the figures of merit (speed, efficiency and pressure) between the initial method and new method is also displayed. It is also possible to enter a different flow rate for the new method and compare the figures of merit.

Notes on the equations used to calculate the new method parameters are given below.

#### Gradient

For the gradient method transfer calculations, instead of analysis time, it is now necessary to enter the timings and mobile phase composition for each segment of the gradient. Additional entry parameters for gradient method transfer are the system dwell volume for both the initial and new system. If there is no isocratic hold at the start of the gradient, then a value of zero should be entered in step 2.

Again notes on the equations used to calculate the key gradient parameters are given below.

### Notes on calculations used in the method transfer calculator

#### Calculation of flow rate

To maintain an equivalent separation when transferring a method, it is important to keep the linear velocity constant between the original and new method while adjusting the flow rate. The linear velocity is related to the flow rate, internal diameter of the column and particle size. A simple equation can be derived to calculate the flow rate ( $F_2$ ) required for the new method:

$$F_2 = F_1 \times (d_{c2}^2 / d_{c1}^2) \times (d_{p1} / d_{p2})$$

$F_1$  - original flow rate (mL/min)

$d_{c1}$  - original column internal diameter (mm)

$d_{p1}$  - original column particle size ( $\mu\text{m}$ )

$d_{c2}$  - new column internal diameter (mm)

$d_{p2}$  - new column particle size ( $\mu\text{m}$ )

#### Calculation of injection volume

Because sub-2  $\mu\text{m}$ -based methods are most often transferred to smaller volume columns, the same injection volume will take up a larger proportion of the new column, possibly leading to band broadening or potentially overloading the column. It is therefore important to scale down the injection volume to match the change in column volume. Once again, a simple equation can be used to calculate the injection volume ( $V_{i2}$ ) required for the new method.

$$V_{i2} = V_{i1} \times (d_{c2}^2 \times L_2 / d_{c1}^2 \times L_1)$$

$V_{i1}$  - original injection volume ( $\mu\text{L}$ )  
 $d_{c1}$  - original column internal diameter (mm)  
 $L_1$  - original column length (mm)  
 $d_{c2}$  - new column internal diameter (mm)  
 $L_2$  - new column length (mm)

### Calculation of gradient profile

Geometrical transfer of the gradient requires calculation of the number of column volumes of mobile phase in each segment (time interval) of the gradient in the original method to ensure that the new calculated gradient takes place over the same number of column volumes, for the new column.

Another simple equation can be derived to calculate each time segment of the gradient in the new method ( $t_{g2}$ ), including column re-equilibration. The equation takes into consideration the volume of each column, the flow rate in the original method, the flow rate in the new method (calculated above) and the time segment in the original method.

$$t_{g2} = t_{g1} \times (V_{02} / V_{01}) \times (F_1 / F_2)$$

$t_{g1}$  - Original gradient (min)  
 $V_{02}$  - New column void volume  
 $V_{01}$  - Original column void volume  
 $F_1$  - Original flow rate  
 $F_2$  - New flow rate

The void volume of the column is the volume that is not taken up by the stationary phase - approximately 68% of the column volume.

### Speed

The speed comparison between the original and new method is simply the ratio of analysis time between the original and transferred method (for isocratic analysis) or the ratio of final gradient time between the original and transferred methods (for gradient analysis).

### Efficiency

The efficiency of a column is known to be proportional to the column length and the inverse of particle size. This means that when transferring a method the efficiency for the new column can be approximated by the following equation:

$$N_2 = N_1 \times (d_{p1} / d_{p2}) \times (L_2 / L_1)$$

One useful outcome of this when replicating a method using a smaller column and particle size is that the efficiency is estimated to remain constant if the ratio of particle size to column length remains constant.

Note that there is a dependence of the efficiency upon the flow rate (or linear velocity through the column). No attempt has been made to adjust the efficiency as a function of flow rate in this calculator.

### Pressure

The pressure drop across a column can be approximated using the following Equation:

$$\text{Pressure Drop (psi)} \sim 250 L \eta F / d_p^2 d_c^2$$

Where

L = Column Length (mm)

$\eta$  = Mobile Phase Viscosity (cP)

F = Flow Rate (mL/min)

$d_p$  = Particle Diameter ( $\mu\text{m}$ )

$d_c$  = Column Internal Diameter (mm)

This equation shows that the pressure drop across the column varies with:

- **The length of the column.** Longer columns have higher pressure drops.
- **The ID of the column.** Narrower columns have higher pressure drops.
- **The diameter of the particles packed within the column.** The smaller the particles the higher the pressure drop. This is a squared relationship and has a significant effect.
- **The flow rate.** A higher flow rate will result in a higher pressure drop.
- **The viscosity of the mobile phase.** Higher viscosities will result in higher pressure drops. Increased temperatures reduce the viscosity, enabling a higher flow rate to be used for an equivalent pressure drop.

The viscosity of the mobile phase is considered to be constant for these method transfer calculations, which results in the relative pressures for the original and transferred method being estimated by the following equation:

$$P_2 = P_1 \times (L_2 \times F_2 / L_1 \times F_1) \times (d_{p1} \times d_{c1} / d_{p2} \times d_{c2})^2$$

### Dwell volume

The dwell volume becomes increasingly important when employing a fast gradient with the small columns typically used with a transferred method because it affects the time taken for the gradient to reach the head of the column. Not taking the dwell volume into account when transferring a gradient method can result in a mismatch in mobile phase composition which can lead to differences in selectivity, particularly for early eluting peaks.

In order to take account of any possible mismatch, a time adjustment is automatically calculated to match the initial isocratic hold for the new method. A negative value for the initial isocratic hold indicates that  $t_0$  for the new method is greater than the geometrically transferred value and it becomes necessary to delay the injection for the time specified.

If the system dwell volume is unknown, it can be estimated using the following method.

- Use methanol for solvents A & B. Add 0.1% acetone to solvent B and prime the lines with the solvents.
- Run B through the detector and adjust the detector to give a full scale absorbance at 260nm.
- Purge the pump with solvent A.
- Run a gradient of 100 %A to 100 %B in 20 min then calculate the time taken to reach 50% absorbance ( $T_{0.5}$ ).
- Determine the difference between ( $T_{0.5}$ ). and 10 min (half the gradient time) and multiply by the flow rate to determine the dwell volume.

**Calculation of equilibration time**

For gradient analysis, the method transfer calculator also provides a suggested column equilibration (or reconditioning) time equal to 10 column volumes.