Scale-Up a purification automatically using the IntelliFlash software “Guide Me” feature

Technical Overview

Purification Scale Up

One of the most common problems associated with drug candidate purification is changing the method to purify more compound – purification scale-up. Agilent IntelliFlash control software, supplied with the Agilent 971-FP Flash Purification System, features a ScaleUp page within the “Guide Me” feature. This allows the user to transfer method details between Agilent SuperFlash columns of different sizes while maintaining compound purity and yield. The user does not need to know the column dimensions nor perform any calculations – these are all supplied automatically by the software.

When scaling an LC purification method up (or down), there are two parameters critical to success. The first is the flow rate used to run the column and the second is the number of column volumes needed for the gradient – the run duration. The columns should be eluted using the same linear velocity and not the same flow rate. The flow rate of the first column and the radii of the two columns are used to calculate the flow rate needed for the second column

Equation 1.
Calculation of flow rate to give the same linear velocity on column 2 as used with column 1

$$F_2 = F_1 \times \left(\frac{r_2}{r_1} \right)^2$$

F_2 = Flow rate needed for column 2 F_1 = Flow rate of column 1

r_2 = Radius column 2 r_1 = Radius column 1

If the two columns are same length, there is no change to the duration of the run when the same linear velocity is used. However, if the columns are of different lengths then a further calculation is required to determine the duration of the run, so that the purification is performed over the same number of column volumes. The length of the column must be considered to calculate the gradient duration.
See Equation 2.

Equation 2.
Calculation of gradient duration when the same linear velocity is used with both columns but the columns have different lengths

$$D_2 = D_1 \times \frac{L_2}{L_1}$$

D_2 = Gradient duration needed for column 2 D_1 = Gradient duration used for column 1

L_2 = Packed bed length of column 2 L_1 = Packed bed length of column 1

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These calculations are straightforward if the dimensions of the two columns are known. An additional operation is required before performing the purification. All of the SuperFlash column dimensions are held within the IntelliFlash software. If these columns are used, the Scale-Up page of the "Guide Me" feature performs this calculation automatically.

The Scale Up option (Figure 1) can be used in two ways:

1. When the method is developed on the 971-FP instrument with a SuperFlash column, select the historical run and then the column to scale up to. Select Apply to automatically load the calculated parameters into a new run.
2. Alternatively, when there is no historical run, select a specified SuperFlash column, flow rate and duration time. Then select the column to scale up to, and click Apply.

Close the window to view the general control screen. This screen displays the new scale-up run conditions.

Scale up in operation

Equations 1 and 2 were used to scale up a separation from a SuperFlash SF25-60 g Si35 to a SuperFlash SF40-115 g Si35 column, and from a SuperFlash SF25-60 g Si35 to a SuperFlash SF25-160 g Si35 column. The dimensions of these three columns are given in Table 1.

Column	id (mm)	Bed length (mm)
SuperFlash SF25-60 g Si35	28.2	166
SuperFlash SF40-115 g Si35	40.6	153
Super Flash SF25-160 g Si35	28.2	458

Table 1
Column lengths and internal diameters used for scale-up calculations.

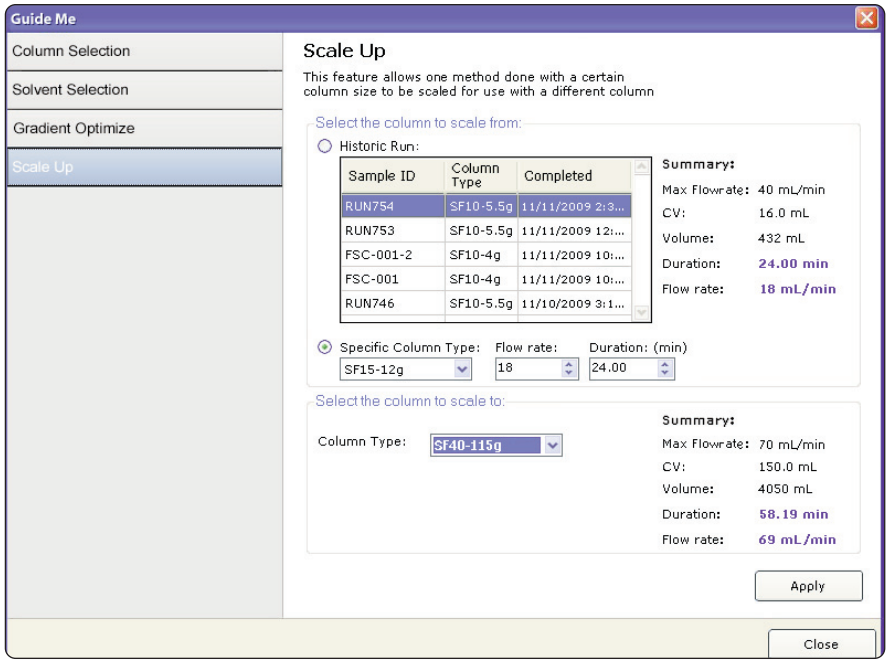

Figure 1
Screen shot of the Scale-Up "Guide Me" page.

Four compounds were prepared in a 50:50 v/v mixture of hexane and ethyl acetate at a concentration of 20 mg/ mL. These compounds were chosen as they are two closely related pairs. The first pair are components of vitamin E, α-tocopherol and δ-tocopherol, and the second are the isomers 2-aminophenol and 3-aminophenol.

As the tocopherols have very little UV activity, an Agilent evaporative light scattering detector was used for peak detection in preference to a UV detector.

Columns:	SuperFlash SF25-60 g Si35, SuperFlash SF40-115 g Si35, SuperFlash SF25-160 g Si35
Eluent A:	Hexane
Eluent B:	Ethyl acetate
Gradient:	0-100%B duration as specified
Flow Rate:	As specified
Detection:	Agilent 385-ELSD (neb = 30 °C, evap = 15 °C, gas = 1.20 SLM) LED 3%

The sample mixture was first run on the SuperFlash SF25- 160 g Si35 column. With the higher efficiency 35 µm media, the four compounds were clearly resolved and visible using the ELSD detector (Figure 2).

When scaling up this separation to a SuperFlash SF40- 115 g Si35 column, both the internal diameter and column length need to be known for both columns. Equation 1 was used to calculate the flow rate needed to operate at the same linear velocity. The flow rate was 69 mL/min for this column. There was a difference in column length so Equation 2 was used to determine the gradient duration of 24 minutes. Figure 3 shows the separation when the SuperFlash SF40-115 g Si35 column was run at a flow rate of 69 mL/min with a gradient duration of 24 minutes. The separations shown in Figures 2 and 3 are very similar.

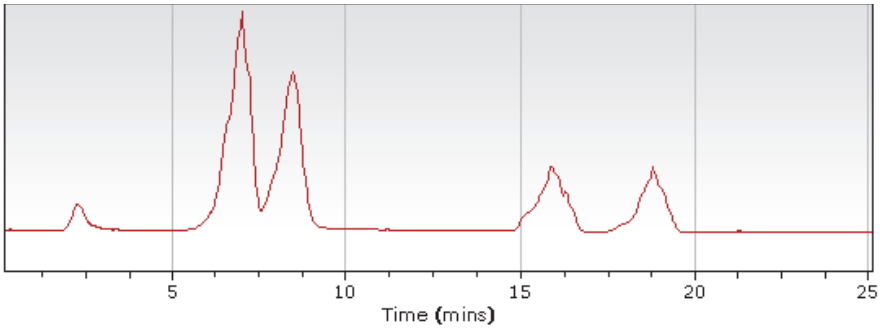

Figure 2
Separation of α-tocopherol, δ-tocopherol, 2-aminophenol and 3-aminophenol using the SuperFlash SF25-60 g Si35 column run at a flow rate of 33 mL/min and with a gradient duration of 26 minutes.

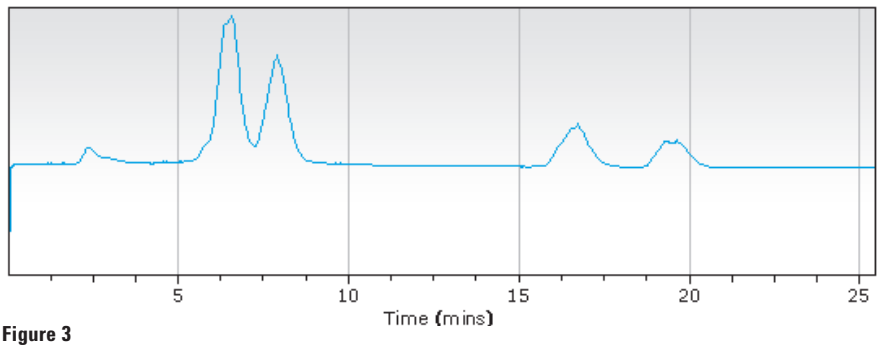

Figure 3
Separation of α-tocopherol, δ-tocopherol, 2-aminophenol and 3-aminophenol using the SuperFlash SF40-115 g Si35 column run at a flow rate of 69 mL/min and with a gradient duration of 24 minutes.

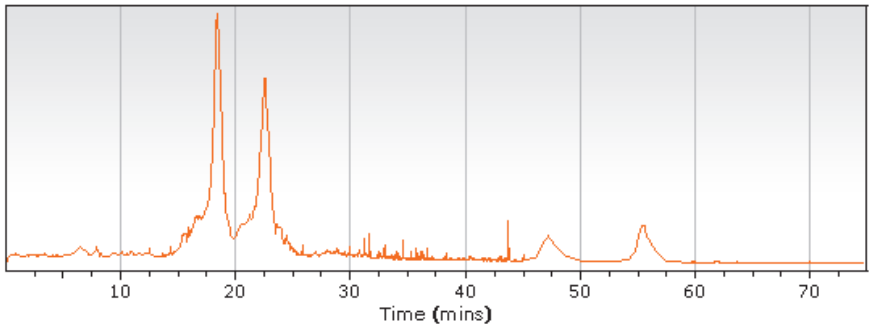

Figure 4
Separation of α-tocopherol, δ-tocopherol, 2-aminophenol and 3-aminophenol using the SuperFlash SF25-160 g Si35 column run at a flow rate of 33 mL/min and with a gradient duration of 72 minutes.

When scaling up from the SuperFlash SF25-60 g Si35 column to a SuperFlash SF25-160 g Si35 column, the internal diameter of the two columns was the same, so the flow rate used was also the same. A longer column length increased the amount of silica. Therefore, in this case only, the gradient duration needed to be changed. The gradient duration was calculated using Equation 2. The chromatogram obtained with the calculated gradient duration is shown in Figure 4.

Comparing the two separations, it was clear that, as expected, there was some improvement in resolution with the longer column but the run time was also much longer.

These data demonstrate that the equations for scaleup can be used successfully for flash purification when the column dimensions are known. If SuperFlash columns are used with the 971-FP instrument and IntelliFlash software, all of the column dimensions are pre-loaded into the software and the Scale Up page of the Guide Me feature can simplify the process.

Knowledge of the column dimensions is not required, nor must calculations be performed to get equivalent performance.

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