A systematic approach to LC method transfer from 4.6 to 2.1mm ID columns

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Abstract

Purpose: To demonstrate a systematic approach to method transfer between 4.6 and 2.1 mm ID columns, with consideration to system set-up and the effect it has on method performance.

Methods: 4 step method to adjust flow rate, injection volume, gradient profile and system set-up.

Results: Results demonstrate savings in solvent consumption, gain in sensitivity and the impact of system volumetric effects on gradient delivery to the column.

Introduction

There are several reasons for reducing the column internal diameter from 4.6 to 2.1 mm. The most common in recent times has been to reduce solvent costs, as mobile phase flow rate through the column is reduced by a factor of 5. Another reason is to improve analysis sensitivity as smaller internal diameter columns provide detection signal enrichment relative to normal bore columns, by eluting solutes in more concentrated chromatographic bands. A third reason is when methods are transferred from UV to MS detection, as large volumes of mobile phase are more difficult to vaporise in the LC/MS interface.

To maintain a consistent assay profile between the original and transferred method it is necessary to scale down flow rate, injection volume and gradient profile in methods that use gradient elution. To obtain the best data it is critical that the LC system is optimized to operate under these conditions. All system components for the assay should be considered. System volume that includes connecting tubing ID and length, injection volume, flow cell volume (in UV) must be minimized, and when running gradients pump dwell volume needs to be minimized.

The work presented in this poster demonstrates a systematic approach to method transfer between 4.6 and 2.1 mm ID columns, with consideration to system set-up and the effect it has on method performance.

Materials & Methods

Instrumentation:
Thermo Scientific Surveyor HPLC system (fitted with 5 cm/ 10 µL flow cell) , Thermo Scientific Accela UHPLC system (fitted with 1 cm / 2 µL flow cell).

Columns:
Thermo Scientific Hypersil GOLD 5 µm, 150 x 4.6 mm, Hypersil GOLD™ 5 µm, 150 x 2.1 mm, Hypersil GOLD 5 µm, 100 x 2.1 mm

Mobile phase: A - H_2O + 0.1% formic acid; B - ACN + 0.1% formic acid
Isocratic method - A : B (80:20)
Gradient: 10 to 50% B in 10 minutes; 100% by 15 minutes.
Temperature: 30 °C; Detection: UV at 270 nm
Flow rate : 1 mL/min or 0.210 mL/min
Injection volume: 20 or 4 µL
Test solution: 1. Uracil; 2. p-Coumaric acid; 3. m-Coumaric acid; 4. o-Coumaric acid

Method transfer
To transfer the method geometrically to the narrower bore column and therefore ensure equivalent chromatography, it is necessary to scale down the flow rate, injection volume and gradient profile:

Step 1. Adjust flow rate
(keep linear velocity constant between original and new method)

$F_2 = F_1 \times (dc_2^2 / dc_1^2)$

F_1 - original flow rate; F_2 - new flow rate (mL/min)
dc_1 - original column ID; dc_2 - new column ID (mm)

Step 2. Adjust injection volume

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

V_{i1} – original injection volume; V_{i2} – new injection volume (mL)
d_{c1} - original column ID; d_{c2} - new column ID (mm)
L_1 - original column length; L_2 - new column length (mm)

Step 3. Adjust gradient profile
If only the column ID has been changed from 4.6 to 2.1mm and column length remains the same then the gradient profile remains unchanged in the new method.
If the column length changes then keep initial and final composition identical and adjust gradient time as follows:

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

t_{g1} – gradient time in original method (min)
t_{g2} - gradient time in new method (min)
V_{01} – original column volume (mL)
V_{02} – new column volume (mL)
F_1 - original flow rate (mL/min); F_2 - new flow rate (mL/min)

This adjustment ensures that the gradient takes place over the same number of column volumes in both columns. If the gradient has several segments then this calculation needs to be performed for each segment to ensure equivalent gradients.

Step 4. Optimise system set-up

4.1 System dispersion

System dispersion or extra-column band broadening can be caused by the volume of the fluidic path within the HPLC instrumentation. This extra-column band broadening has a detrimental effect on chromatographic performance, and is particularly more significant when working with short narrow bore columns, as the relative amount of extra-column volume compared to the volume occupied by the column becomes more significant. It is, therefore, critical to minimize extra-column dispersion when working with these columns.

4.2. Pump dwell volume

The pump dwell volume is extremely important when running fast applications using gradient elution. It affects the time it takes for the gradient to reach the head of the column.

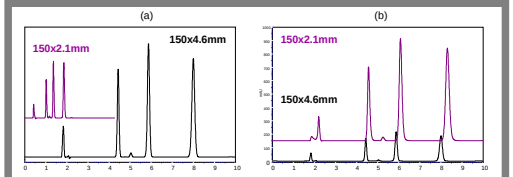
Results

No adjustment of the flow rate when changing to the 2.1mm ID column results in a linear velocity through the column approximately 5 times higher than in the 4.6 mm ID column, as demonstrated by shorter analysis time in Figure 1a.
No adjustment of the injection volume when changing to the 2.1mm ID column results in signal intensity which is approximately 5 times higher than in the 4.6 mm ID column, as demonstrated in Figure 1b.

The chromatography obtained by correctly scaling down the flow rate and injection volume for an isocratic method is demonstrated on Figure 2.

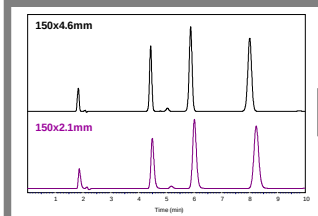
The effect of pump dwell volume on gradient separations is demonstrated on Figure 3. A standard system with 800 µL dwell volume adds approximately 3 minutes to the elution time on a 2.1mm ID column in relation to the elution times on a 4.6mm ID column (Figure 3a). Method sensitivity is improved by a factor of 2 with the 2.1mm ID column as shown in Figure 3b.

FIGURE 1. Isocratic method run on 150x4.6mm and 150x2.1mm columns keeping flow rate constant (a) and injection volume constant (b).



STEP 1 and 2: Adjust flow rate and injection volume

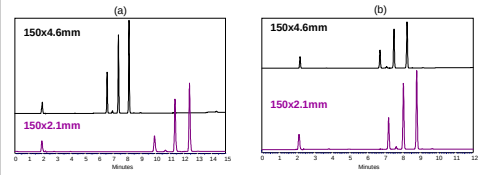
FIGURE 2. Isocratic method run on 150x4.6mm and 150x2.1mm columns with same linear velocity and injection volume proportional to column cross section.



	Flow rate (mL/min)	Injection volume (µL)
4.6 mm ID	1.0	20
2.1 mm ID	0.21	4

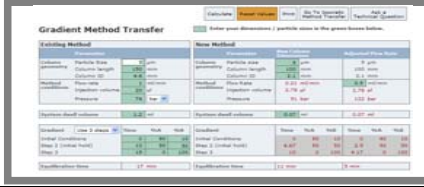
STEP 3 and 4: Adjust gradient and system set-up

FIGURE 3. Effect of pump dwell volume on gradient method. (a) Method run on HPLC system with pump dwell volume of 800 µL; (b) method run on HPLC system with pump dwell volume of 65 µL.



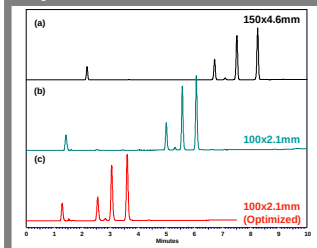
Transfer method to a shorter column

FIGURE 4. Gradient method transfer calculator (http://www.hplctransfer.com/ [1]). Transfer of gradient method to a shorter column (from 150 x 4.6mm to a 100 x 2.1mm).



The method was transferred to a shorter column, as resolution between all the sample components is high. The gradient was adjusted by using an online method transfer calculator as shown in Figure 4. The separation obtained is compared to the original gradient method on the 150 x 4.6 mm column in Figure 5 a and b. In Figure 5c the optimization of the gradient to shorten the run time is also shown.

FIGURE 5. Method run on a 100 x 2.1mm column to reduce run time. (a) Original gradient method on 150x4.6mm; (b) Gradient and conditions as described in "New Method" in Figure 4; (c) Optimized gradient (c). Summary of solvent, time and sample savings.



Optimized gradient:

Time (min)	%B
0	25
5	50
7.5	10

	150x4.6mm	150x2.1mm	100x2.1mm (optimized)
Run time (min)	15	15	7.5
Flow rate (mL/min)	1.0	0.21	0.21
Solvent used (mL)	30	6.3	2.1
Injection volume	20	4	3

Conclusions

- To ensure equivalent chromatography when transferring a method to a narrower bore columns it is necessary to scale down flow rate, injection volume and gradient profile for gradient elution methods;
- In gradient methods the pump dwell volume has a significant impact on the method transferability, as it adds to the time for the gradient to reach the column;
- System volume which adds to band dispersion needs to be minimized when working with narrow bore columns.
- Reducing the column internal diameter from 4.6 to 2.1mm reduces solvent consumption and sample consumption by a factor of 5.
- Method sensitivity is improved with narrow bore columns.
- Optimization of gradient speeds up analysis.

References

1) http://www.hplctransfer.com/

For additional information, please visit our Chromatography Resource Centre which can be found at:
www.thermoscientific.com/chromatography

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