Transfer of Methods in LC and UHPLC, What Calculations do I need ?

Ken Butchart, Mark Woodruff. • Fortis Technologies Ltd, 45 Coalbrookdale Road, Cheshire, CH64 3UG, UK.

Introduction

Much is made of the ability of UHPLC to speed up the analysis of samples, improve high throughput screening, or to develop new methods quickly which can be scaled to/or from traditional HPLC.
In order to perform method transfer there are several 'method development' calculators available to help in making appropriate changes to column dimension, flow rate and gradient conditions. If done properly the overall method time will reduce but resolution and selectivity of solutes will remain constant or indeed improve.

In this poster we discuss the equations involved so that the analyst has a fundamental understanding of the calculations and can appreciate them in context of the method development taking place. Variables in method development should be understood so that an effective method can be produced quickly and efficiently.
We believe that understanding of the fundamental chromatographic equations is necessary in an analysts training so chromatographic method integrity is maintained.
We show how the changes suggested with the calculations lead to savings in both time and solvent for the method. We discuss criteria that are necessary such as UHPLC columns that possess the same physical characteristics as their HPLC counterpart.

Column Efficiency

In order to move a HPLC separation to a shorter UHPLC column there is a need to maintain column plate count, "Separating Power". In figure1 we show the ability to maintain the same separation power.

FIGURE 1. Column 'Separating Power'

Column Length	Efficiency of 5µm	Efficiency of 3µm	Efficiency of 1.7µm
250	22000		
150	12700	16800	26460
100	8300	10700	21000
50	4000	6000	11200
30		3200	7000
20			3000

Increase Speed

Save Solvent

8 fold

So for example if you move from a 5µm 150x4.6mm to a 1.7µm 50x2.1mm the equivalent separation should be achieved but a several fold improvement in analysis time will be achieved. In Figure 2 we now have very peaks and excessive resolution as a result of the initial move to a UHPLC column. The method can now be improved by moving to a higher flow rate, this lowers our retention time to 2min from the initial 20min HPLC run time.

Calculations

The first thing that we need to change to scale down to a UHPLC column is flow rate, this keeps the linear velocity across the methods identical, to do this we use:

New Flow Rate = (diameter of new column / diameter of old column)^2 x old flow rate

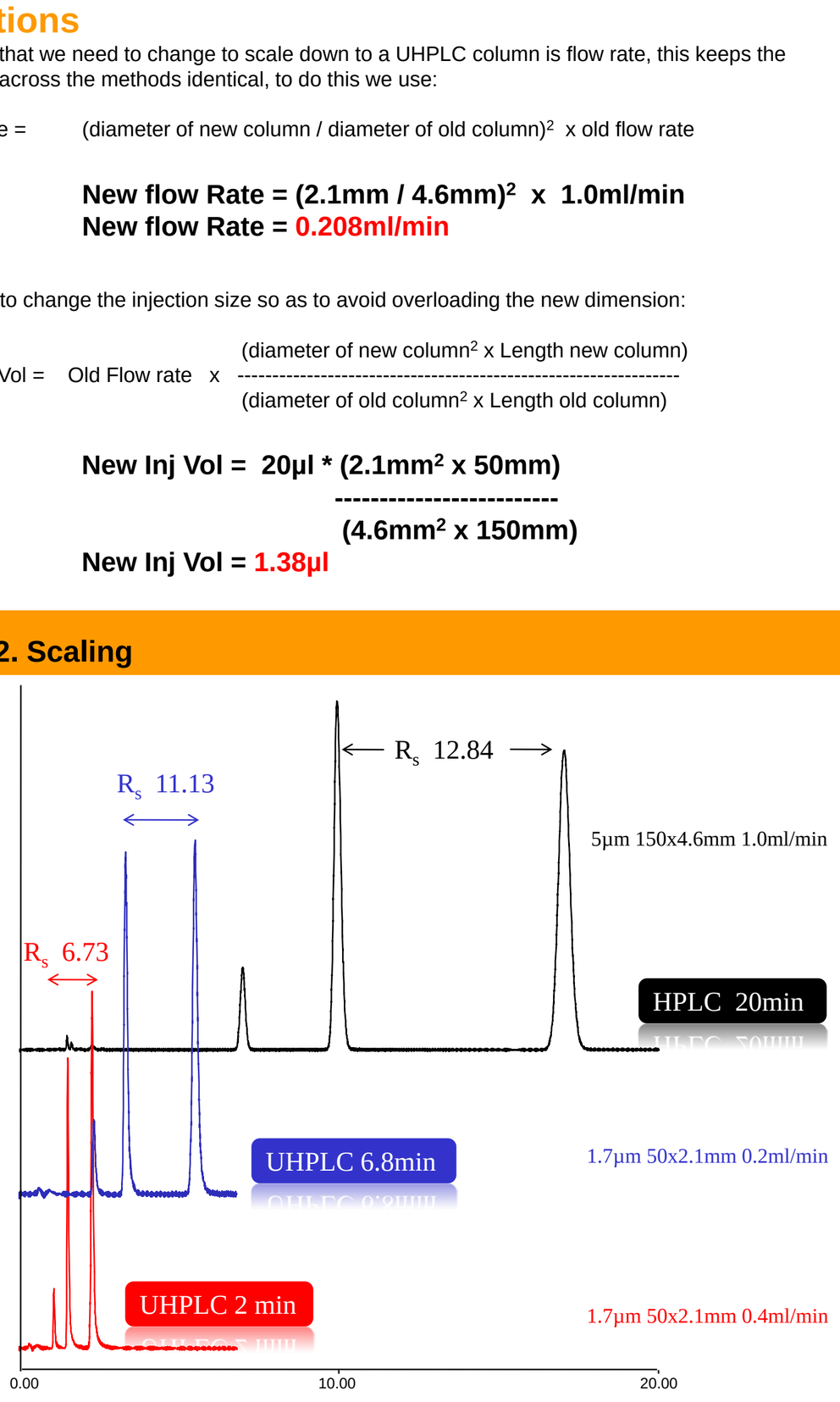
Example: **New flow Rate = (2.1mm / 4.6mm)^2 x 1.0ml/min**
New flow Rate = 0.208ml/min

Next we need to change the injection size so as to avoid overloading the new dimension:

$$\text{New Injection Vol} = \text{Old Flow rate} \times \frac{(\text{diameter of new column}^2 \times \text{Length new column})}{(\text{diameter of old column}^2 \times \text{Length old column})}$$

Example: **New Inj Vol = 20µl * $\frac{(2.1\text{mm}^2 \times 50\text{mm})}{(4.6\text{mm}^2 \times 150\text{mm})}$**
New Inj Vol = 1.38µl

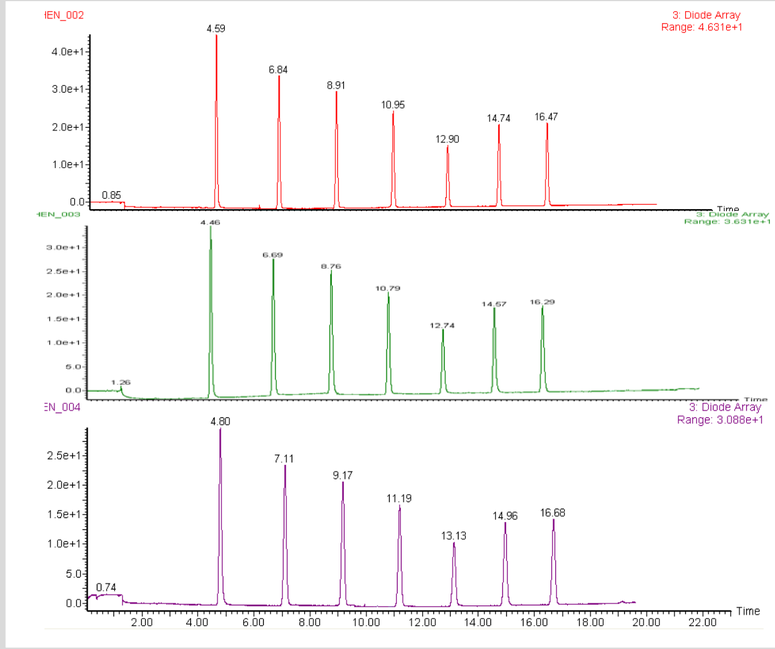
FIGURE 2. Scaling



Critical Consideration – Phase Chemistry Scalability

Using the calculations outlined so far we are assuming that the HPLC phase is remaining constant in terms of chemistry, surface area and %Carbon. If this is the case then scalability of methods will be straightforward. We have already outlined the problems that can occur if the physical characteristics of the stationary phase change in previous work[*1,2]. In Figure 3 we show that the Fortis 1.7µm, 3µm and 5µm particles all provide the exact same chromatography so if the method is transferred from UHPLC to analytical or preparative scale then the same method will be applicable. If the chemistry, surface area or %C do change then the scalability of the method is compromised.

FIGURE 3. Scalability of Surface Area and %Carbon



1.7µm Fortis™ C18
380m^2/g 17%Carbon
N= 178,020

3 µm Fortis ™ C18
380m^2/g 17%Carbon
N= 130,191

5 µm Fortis ™ C18
380m^2/g 17%Carbon
N= 88,708

Gradient Changes

In order to change our gradient we must aim to keep the slope and the start point the same but lower the time the gradient runs in.

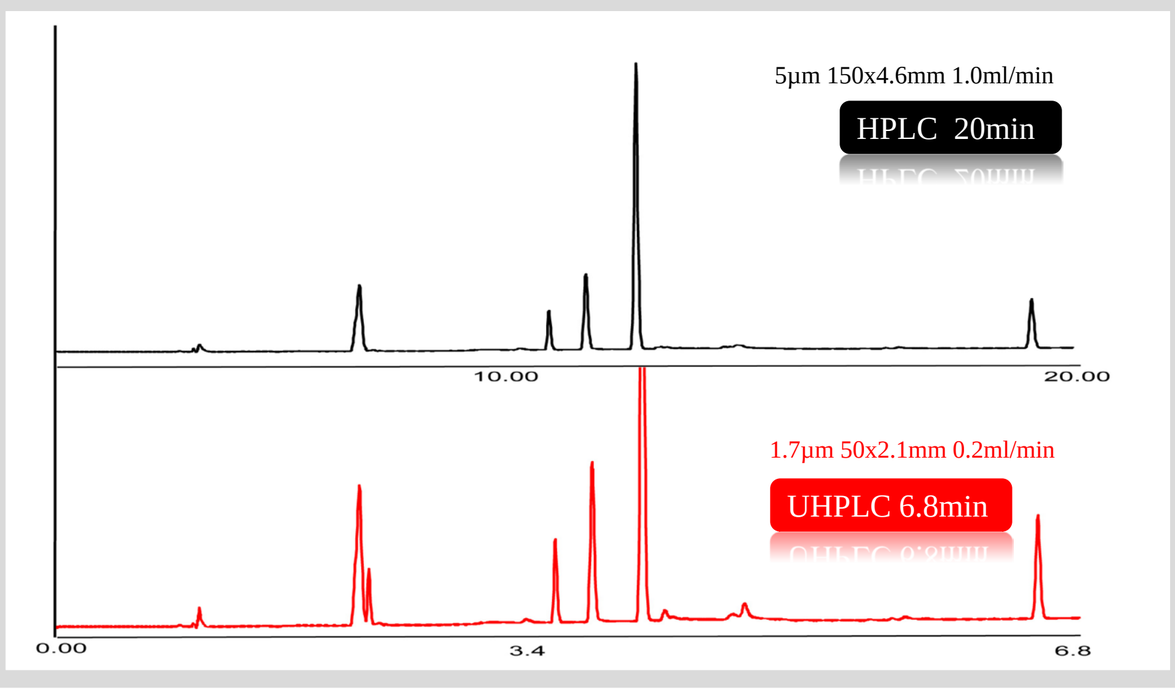
Altering the gradient time, retains the same linear gradient and slope, but reduces the run time:

$$\text{New Gradient time} = \text{Old gradient time} \times \frac{(\text{Old Flow})}{(\text{New Flow})} \times \frac{(\text{New Diameter}^2)}{(\text{Old Diameter}^2)} \times \frac{(\text{New Length})}{(\text{Old Length})}$$

Example:New Grad time = 20 min x $\frac{(1.0\text{ml/min})}{(0.2\text{ml/min})}$ x $\frac{(2.1\text{mm}^2)}{(4.6\text{mm}^2)}$ x $\frac{(50\text{mm})}{(150\text{mm})}$

New Gradient time = 6.95min

FIGURE 4. Gradient transfer



USP Compendial Methods Changes - 621

The latest S*timuli**3 article of Chromatography (621) aims to increase the options currently available to adjust HPLC column length and particle size to achieve separation power to at least equivalent to that used in the original procedure.

- Change Column length ± 70%
- Change Column Diameter ± 25%
- Change Particle Size – Reduce by up to 50%

By allowing these changes the United States Pharmacopeia (USP) is hoping to provide flexibility in developing analytically equivalent procedures that decrease analysis time and solvent consumption, without requiring revalidation of the method.

Conclusion

Whilst calculators are available in order to help us achieve method transfer from analytical to UHPLC.
It is also wise for the analyst to understand the fundamental equations that are involved in the transfer process.
Initially we can alter the mobile phase flow rate and the sample injection volume, without changing the method resolution. Then if necessary we can alter the UHPLC method still further with increased flow rate or higher organic concentration to decrease run time whilst still maintaining a more than acceptable resolution between critical pairs
Understanding and consideration of the above variables will lead to a more robust method being designed.

*1 K.Butchart et.al) . **International Labmate (2007)**, Vol. XXXII Issue V
*2 K.Butchart, G.Foster, D.Temesi, **Chromatography Today**, 1, 3-7, (2007)
*3 **Pharmacopeial Forum** Vol.35(6) [Nov-Dec 2009]

Strength in Technology...

www.uhplccolumns.com/calculator

Fortis™ Technologies Ltd.